Eunice Kennedy Shriver
NICHD
National Institute of Child Health & Human Development

16 May 2011

Dear Fellows and Graduate Students,

Welcome to the Seventh Annual NICHD Fellows Retreat! Once again we take a much needed respite from our daily routines to convene at the scenic Airlie Center, tucked away in the rolling hills of the Virginia countryside. This is *your* time each year to join together with your peers away from the confines of the lab to talk science and share ideas, relax, introduce yourself to new faces, and celebrate our diverse community of trainees. Make the most of it! It is my hope that you will leave the retreat with new friends, perspectives, and a refreshed state of mind.

A dedicated group of fellows and graduate students worked very hard to create this retreat, with all of you in mind. The members of the steering committee, who provided all of the creativity, ideas, advice, and legwork required for the successful coming together of this event, consisted of: Cheryl Bolinger Ph.D., Melissa Crocker M.D., Lina Gugliotti Ph.D., Jana Kainerstorfer Ph.D., Kara Lukasiewicz Ph.D., Fiona Mitchell Ph.D., and Jason Riley Ph.D. Please be sure to thank them by attending all of the sessions. On behalf of the committee and the NICHD community, I'd like to extend a heart-felt thank you all of our participants, who have taken time from their work to join us and make this event very special: NICHD leadership, our keynote speakers, NICHD alumni career panelists, and NICHD investigators.

As in years past, this meeting could not have been possible without the dedication and hard work of Ms. Brenda Hanning, Director of the NICHD Office of Education. Brenda truly cares for the trainees at NICHD, and goes over the top daily to bring us the very best training program possible. When other institutes now host their fellows retreats on campus, or have eliminated them altogether, Brenda has budgeted carefully to preserve our traditional "green" retreat. She feels that the retreat gives us a necessary networking opportunity, the chance to plan and execute a meeting in its myriad details, and connections to NICHD alumni who have identified stimulating careers for themselves. I couldn't agree more, and having attended the past two retreats, I know that all of attendees feel similarly and appreciate this special time. We also deeply appreciate the generous support we receive from our Scientific Director, Dr. Constantine Stratakis, and Dr. Alan Guttmacher, the NICHD Director. We are very lucky to have leadership that cares deeply about our success, in whatever we choose to pursue with the excellent training we receive here.

We are very thankful for Nicki Jonas, who does such a wonderful job with designing our poster and program booklet, and Loc Vu, who is responsible for the retreat website.

Finally, thanks to all of you for taking time from your lab work to come here to share your research and show your support with the NICHD community.

It's been an absolute pleasure and honor serving the NICHD fellows community as the chair of this year's retreat steering committee, and I sincerely hope you enjoy!

Cheers,

Kristofor Langlais, Ph.D.
Chair, Seventh Annual NICHD Fellows Retreat Steering Committee

Credit: Cover art provided by Preethi Chandran, Section on Tissue Biophysics and Biomimetics. Atomic Force Imaging shows that DNA forms complex nanostructures with multivalent ions. The interaction can be modulated to obtain virus-like particles which serve as nanomedicines that deliver a anti-retroviral genes to compromised cells. Each image area is 1um × 1um.

SEVENTH ANNUAL MEETING OF POSTDOCTORAL, CLINICAL, AND VISITING FELLOWS AND GRADUATE STUDENTS

Airlie Center • Warrenton, Virginia • May 16 & 17, 2011

program

MONDAY, MAY 16, 2011

3:00 – 5:00 p.m.	REGISTRATION
5:00 – 6:00 p.m.	KEYNOTE ADDRESS *Moderator, Kristofor Langlais, Ph.D.* "Science, Society, and the Social Contract" Ann C. Bonham, Ph.D. Chief Scientific Officer Association of American Medical Colleges Washington, DC
6:15 – 7:15 p.m.	DINNER
7:15 – 8:45 p.m.	POSTER SESSION 1 (ODD NUMBERS) *Outdoors in the pavilion*

TUESDAY, MAY 17, 2011

6:30 – 7:30 a.m.

NATURE WALK: FIELDS AND PONDS *(OPTIONAL)*

7:30 – 8:30 a.m.

BREAKFAST

8:30 a.m.

WELCOME

Kristofor Langlais, Ph.D., Chair, Annual Meeting Steering Committee
Constantine A. Stratakis, M.D., D.(med.)Sci., Acting Scientific Director, NICHD

8:50 a.m.

SCIENTIFIC PERSPECTIVES

Moderator, Jason Riley, Ph.D.

"Manipulation of Host Cell Signaling Proteins by *Legionella pneumophila*"
Matthias Machner, Ph.D.
Unit on Microbial Pathogenesis
Cell Biology and Metabolism Program, NICHD

"Making the Invisible Visible with Diffusion MRI"
Peter J. Basser, Ph.D.
Laboratory of Tissue Biophysics & Biomimetics
Director, Program on Pediatric Imaging & Tissue Sciences, NICHD

10:00 a.m.

Coffee break & check out

10:30 a.m. – 12 noon

POSTER SESSION 2
(EVEN NUMBERS)

Outdoors in the pavilion

TUESDAY, MAY 17, 2011
(continued)

12:00 – 1:00 p.m.

TUESDAY KEYNOTE ADDRESS

Moderator, Kara Lukasiewicz, Ph.D.

"Improbable Research and the Ig Nobel Prizes"
Marc Abrahams, Ph.D.
Editor & Co-founder, *Annals of Improbable Research*
Boston, MA

1:00 – 2:00 p.m.

LUNCH

2:00 – 3:15 p.m.

CAREER Q&A TABLES

Moderators, Drs. Melissa Crocker, Lina Gugliotti
Select 3 rotations, 20 minutes each, from:

1. Research, Academe/NIH
   - Xiangyun Qiu, Ph.D., Dept. of Physics, George Washington University
2. Science Policy
   - Christine Torborg, Ph.D., NINDS
3. Grants & Scientific Administration
   - Melissa Cunningham, Ph.D., CDMRP, Ft. Detrick
4. Consulting
   - Zigurts Majumdar, Ph.D., Booz Allen Hamilton
5. Food and Drug Adminsitration (FDA)
   - Newton Woo, Ph.D., FDA
6. Research, Pharma/Private Sector
   - Jeanne Fringer, Ph.D., U.S. Pharmacopeia

TUESDAY, MAY 17, 2011
(continued)

3:15 – 4:00 p.m.

FELLOW PRESENTATIONS 1-3

Moderator, Jana Kainerstorfer, Ph.D.

"Hindered Diffusion in Polymeric Solutions Studied by Fluorescence Correlation Spectroscopy"

Silviya Zustiak, Ph.D., NICHD Program in Physical Biology

"Modulation of DNA Condensation by Ion Valence for Nanomedicine Applications"

Preethi Chandran, Ph.D., NICHD/NIBIB Fellow

"An N-terminal Truncated Carboxypeptidase E Splice Isoform Induces Tumor Growth and Is a Biomarker for Predicting Future Metastasis in Human Cancers"

Saravana Murthy, Ph.D., NICHD Program in Developmental Neuroscience

4:00 – 4:10 p.m.

BREAK

4:10 – 5:00 p.m

FELLOW PRESENTATIONS 4-6

Moderator, Cheryl Bolinger, Ph.D.

"Expression Profiling of Autism Candidate Genes during Human Brain Development Implicates Central Immune Signaling Pathways"

Mark Ziats, MD-PhD student, Cambridge University/NIH
Laboratory of Clinical Genomics, NICHD

"Analysis of DNA Re-replication Dynamics: What Can We Learn about Origins of Replication in Mammals?"

Christelle de Renty, Ph.D., NICHD Program in Genomics of Differentiation

"Generation of Induced Pluripotent Stem Cells Is Regulated by Mitochondria within the Somatic Cell of Origin"

Kevin Francis, Ph.D., NICHD Program in Genomics of Differentiation

SEVENTH ANNUAL MEETING OF POSTDOCTORAL, CLINICAL, AND VISITING FELLOWS AND GRADUATE STUDENTS

May 16 & 17, 2011

speaker profiles

MARC ABRAHAMS, PH.D.

Marc Abrahams, applied mathematician and entrepreneur, is the editor and co-founder of the science humor magazine *Annals of Improbable Research*. He writes about research to make people laugh, and then think. His team comprises the very same people who from 1955-1994 founded and edited the *Journal of Irreproducible Results*. In 1994, when the journal's publisher decided to abandon the magazine, they decided to abandon that publisher. Unable to get use of the old name, they started the new publication, the *Annals of Improbable Research*. And yes, they say, "we are *AIR*heads." Named the "Puck of Science," Abrahams is master of ceremonies for the Ig Nobel Prizes, awarded yearly in a gala ceremony at Harvard University. A prolific writer, Abrahams is author of *The Man Who Cloned Himself, Why Chickens Prefer Beautiful Humans*, and *The Ig Nobel Prizes*, and he has authored the librettos for 15 science mini-operas. See, too, www.improbable.com.

PETER J. BASSER, PH.D.

Dr. Basser received his Ph.D. from Harvard University and joined the NIH right after. Within NICHD he is the director of the Program on Pediatric Imaging and Tissue Sciences, where his research focuses on developing new quantitative imaging biomarkers that are both sensitive and selective, and can be used in screening, diagnosis, prognosis assessment, and even therapy, primarily relating to neurodevelopment. Additionally, the Program aims to transfer these new methodologies "from bench to bedside" and to various biomedical research communities. Those technologies include diffusion tensor NMR and MRI (DTI), which Dr. Basser co-invented. For two decades Dr. Basser has been working on translating this novel technology to the clinic and making it a basic research tool to probe brain architecture and organization. DTI has had a transformative impact in radiology, neurosciences, and even psychiatry, helping scientists and physicians study the structure and organization of the normal, developing, and diseased brain.

ANN C. BONHAM, PH.D.

Dr. Bonham is the Chief Scientific Officer at the Association of American Medical Colleges in Washington, DC. She directs the AAMC's array of programs that support all aspects of research and training. As the primary AAMC contact for external research organizations, Dr. Bonham addresses policy issues affecting research through engagement with key officials in the public and private sectors. Dr. Bonham also works closely with AAMC constituents to address their research needs and represents the association on the national stage in forums dealing with research policy and administration.

Prior to joining the association, Dr. Bonham served as executive associate dean for academic affairs and professor of pharmacology and internal medicine at the University of California, Davis, School of Medicine, where she oversaw the school's research, undergraduate medical education, and faculty academic programs. Dr. Bonham received her doctoral degree in pharmacology from the University of Iowa College of Medicine and completed a postdoctoral fellowship at Northwestern University School of Medicine.

speaker profiles (continued)

MELISSA D. CUNNINGHAM, PH.D.

Dr. Cunningham transitioned from the bench to grant administration in June 2010 when she joined the Congressionally Directed Medical Research Programs (CRMRP) with the Department of Defense. As a Science Officer in the Prostate Cancer Research Program (PCRP), she manages grants awarded to prostate cancer researchers and is involved with a variety of other efforts to help enhance the research funded by CDMRP, such as promoting advanced technology development. Before joining CDMRP, Dr. Cunningham trained as a postdoctoral fellow at the National Institute of Child Health and Human Development, National Institutes of Health. During her fellowship Dr. Cunningham discovered her interest in grants management after working part-time with the Program Office in NICHD's Intellectual and Developmental Disabilities (IDD) branch.

Dr. Cunningham graduated from Towson University, summa cum laude, in 2001, with a B.S. in Biology, and earned her Ph.D. in Biochemistry, Microbiology and Molecular Biology from the Pennsylvania State University in 2006. Her areas of expertise include epigenetics, gene regulation, and bioinformatics in *Saccharomyces cerevisiae* and *Drosophila melanogaster.*

CHRISTELLE DE RENTY, PH.D.

Dr. de Renty received her Ph.D. in Biology and Health from the University of Montpelier, France, where she studied DNA replication mechanisms and dynamics by using yeast *Saccharomyces cerevisiae* as a model system. This was the opportunity to learn a novel and state-of-the-art technique—DNA DNA combing—which allows a high density analysis of single DNA molecule. She joined NICHD in 2009 as a postdoctoral fellow and uses her expertise to study DNA replication and re-replication dynamics in cancer cells.

KEVIN FRANCIS, PH.D.

Dr. Francis received his B.S. from Marshall University and his Ph.D. from the Medical University of South Carolina in 2009. His dissertation research examined mechanisms to promote the survival and neural differentiation of mouse and human embryonic stem cells. In the summer of 2009, he joined the laboratory of Dr. Heiner Westphal in the Program on Genomics of Differentiation at NICHD as an IRTA Postdoctoral Fellow. Dr. Francis is currently researching mechanisms regulating induced pluripotent stem cell formation, while also applying this technology to disease models of human neurodegeneration.

JEANNE FRINGER, PH.D.

Dr. Fringer received a B.S. in Biochemistry from the University of Rochester and a Ph.D. in Biochemistry and Molecular Biology from the University of Texas Southwestern Medical Center in Dallas, Texas. She did her postdoctoral training at NICHD in the laboratory of Thomas Dever, Ph.D., working on translation initiation.

Currently, she works at US Pharmacopeia in the Biologics and Biotechnology laboratory in Rockville, MD. Her job is to develop and validate methods which characterize reference standards for drugs. One of her main areas of interest is developing cell-based assays that measure the potency of drugs such as hormones and growth factors.

MATTHIAS MACHNER, PH.D.

Dr. Machner received his Ph.D. from the Carolo-Wilhelmina Technical University of Braunschweig, Germany, for his studies on virulence factors from the bacterium *Listeria monocytogenes*. He did postdoctoral training with Ralph Isberg at Tufts University School of Medicine in Boston, MA, where he began his studies on Legionnaires' disease, a severe pneumonia caused by the bacterium *Legionella pneumophila*. Dr. Machner joined NICHD in 2008 as Head of the Unit on Microbial Pathogenesis. His group combines biochemical, genetic, and imaging techniques to identify and characterize molecular processes that allow *L. pneumophila* to survive and replicate within host cells.

speaker profiles (continued)

ZIGURTS MAJUMDAR, PH.D.

Ziggy Majumdar is a native of Brooklyn, NY, grew up in Westchester County, and got his B.S. and Ph.D. degrees in physics from the University of Pennsylvania and University of Illinois at Urbana-Champaign, respectively. He came to NICHD in June 2006 as an IRTA postdoctoral fellow working with Bob Bonner in the Laboratory for Integrative and Medical Biophysics. A large part of his work at NIH was collaborative with the National Eye Institute in developing spectral imaging methods to quantify and detect early stages of retinal eye disease, such as age-related macular degeneration. Other work involved dabbling with the application of optical technologies to clinical problems, such as high-throughput, high-resolution laser-based microdissection of tissue and programmable lighting for healthier home and workplace environments. In February 2009 he began work as a Senior Consultant at Booz Allen Hamilton and was promoted to Associate in December 2009. He applies his scientific expertise for government clients that need technical support, which includes developing new research program concepts, reviewing research, and collaborating with researchers and potential technology "end-users" to help bridge the gap between ongoing research and government needs in sectors such as the Department of Defense and Health.

SARAVANA MURTHY, PH.D.

Dr. Murthy is a research fellow in the laboratory of Dr. Peng Loh, in NICHD's Section on Cellular Neurobiology. He studies a novel splice variant of Carboxypeptidase E, ΔN-CPE and its mechanism in epigenetic gene regulation. CPE-ΔN induces tumor metastasis and is a powerful biomarker for predicting future metastasis and recurrence in several human cancers. CPE is also shown to have neuroprotective roles when hippocampal neurons are challenged to oxidative stress, so he is also studying the role of CPE during glucocorticoid mediated stress response. Dr. Murthy completed his master's degree at Bangalore University, India and for his doctorate went to Max Planck Institute, Goettingen, Germany to work on characterization of SK2 channels and its splice variants. He came to the US as a visiting PhD student at the University of Hawaii and later joined Dr. Loh's lab as a visiting fellow; since then he has been working on the epigenetic mechanism of ΔN-CPE.

XIANGYUN QIU, PH.D.

Xiangyun Qiu went to the University of Science and Technology of China for his Bachelor's degree in physics. He then left China in 1999 to study for his PhD in condensed matter physics at Michigan State University. He made a shift to biophysics as a postdoctoral fellow at Cornell University, working with Professor Lois Pollack, and then moved to NICHD as a research fellow with Dr. Adrian Parsegian. Since 2010, he has been assistant professor of physics at the George Washington University. His general research interest is the biophysics of nucleic acids and the interface between biomolecules and minerals. The primary experimental technique in his lab is small, medium, and wide angle x-ray scattering.

CHRISTINE TORBORG, PH.D.

Dr. Torborg is currently a Health Policy Analyst in the Office of Science Policy and Planning at the National Institute of Neurological Disorders and Stroke. She received her BA in Biology and Chemistry from Gustavus Adolphus College in St. Peter, MN in 2000 and did her graduate work at the University of California, San Diego. While there, she received an NSF Graduate Research Fellowship to study the role of spontaneous retinal activity in the development of the connections between the retina and the brain in mice. After receiving her PhD in 2004, Dr. Torborg took a postdoctoral position in the laboratory of Dr. Chris McBain in the National Institute of Child Health and Human Development, where she received a PRAT fellowship to study inhibitory interneurons in the hippocampus of juvenile rodents. Starting in the spring of 2010, she did "detail" rotations in two offices in the National Institute of Neurological Disorders and Stroke, and was hired for a full-time position there in October of 2010.

PREETHI SAINI, PH.D.

Dr. Saini is an NIBIB/NICHD postdoctoral fellow who likes to dwell in the murky waters bordering biology and engineering. She got her PhD at the University of Minnesota and did postdoctoral stints at Columbia University, NY, and UC Berkeley, CA. In the process she has studied the filament macromolecules and arrangements, which give structural integrity to the extracellular and intracellular matrix, from a multi-scale modeling and

speaker profiles (continued)

experimental perspective. She considers herself a civil engineer entrapped by the intricacies of cell and tissue architecture. During her time at the NIH, she has entered the sacred confines of the cell nucleus. She is, among other things, looking at the complex structures formed by DNA and polyvalent ions, and their application in nanomedicine.

NEWTON WOO, PH.D.

Dr. Woo is a Pharmacology/Toxicology reviewer in the FDA who evaluates nonclinical data to assess the safety and risk-benefit of various pharmaceutical compounds. Born and raised in Canada, Dr. Woo received his BSc (Pharmacology) from the University of British Columbia and his PhD (Neurophysiology) from the University of Alberta studying various forms of synaptic plasticity in the mammalian brain. He continued his training as a Visiting Fellow at the National Institute of Child Health and Human Development under the guidance of Dr. Bai Lu, where he discovered novel roles for neurotrophins in several forms of synaptic plasticity. In his current FDA position, he applies his education and neuroscience expertise in reviewing animal data to assess and extrapolate safety and efficacy of various therapeutic agents as well as enforce regulatory nonclinical standards to ensure the safe use of newly developed drugs in the clinic.

MARK ZIATS

Mark Ziats graduated from Clemson University with a Bachelor's degree in Biochemistry in 2007. He then spent a year as an NIH Academy fellow in the laboratory of Dr. William Gahl at NHGRI before beginning the NIH-University of Cambridge MD-PhD Graduate Partnership Program in 2008. He completed his first two years of medical school at Baylor College of Medicine and is now pursuing his PhD work in the laboratories of Dr. Owen Rennert at NICHD and Dr. Azim Surani at Cambridge. His thesis work is investigating gene expression, long non-coding RNAs, genomic imprinting, and the role of glia in normal developmental and neurodevelopmental disorders like Autism.

SILVIYA PETROVA ZUSTIAK, PH.D.

Dr. Zustiak received a doctoral degree in Chemical and Biochemical Engineering from University of Maryland Baltimore County and a BS/MS degree in bioelectrical engineering from Technical University, Sofia, Bulgaria. Her masters' research involved the design of a hybrid biosensor for ecological purposes, and her doctoral research was centered on developing and testing synthetic biomaterials for neural tissue engineering. Silviya accepted an IRTA position at NICHD in the beginning of 2010, in the Laboratory of Integrative and Medical Biophysics under Dr. Ralph Nossal. Her postdoctoral research is focused on building a 3D in-vitro platform for cancer drug screening, as well as the application of spectroscopy techniques for the elucidation of transport properties in complex systems. Her professional goal is to obtain a tenure-track faculty position in a Chemical, Biomedical, or Bioengineering Department.

poster presentations

ODD NUMBERS

Monday, May 16, 2011

7:15 p.m. – 8:45 p.m.

EVEN NUMBERS

Tuesday, May 17, 2011

10:30 a.m. – 12 noon

categories

Presenting fellows' names are listed in **bold**.

1 De-AMPylation of the small GTPase Rab1 by the pathogen *Legionella pneumophila*

Ramona Aldea, Yang Chen, Matthias Machner

Upon inhalation of contaminated aerosols, the intracellular pathogen *Legionella pneumophila* can infect human alveolar macrophages causing a severe pneumonia known as Legionnaires' disease. This pathogen translocates a large number of effector proteins into the host cell that coordinate establishment of a replication vacuole called the *Legionella*-containing vacuole (LCV). *L. pneumophila* exploits host cell vesicle transport by manipulating the small GTPase Rab1, a key regulator of ER to Golgi vesicle transport. The *L. pneumophila* effector protein SidM recruits Rab1 to the LCV early during infection and it activates Rab1 through GDP/GTP exchange. Rab1 is subsequently inactivated by the effector protein LepB and removed from the LCV. Recently, it was shown that SidM also AMPylates activated Rab1 through the covalent addition of adenosine monophosphate. In vitro, AMPylated Rab1 is protected from inactivation by the *L. pneumophila* protein LepB. This finding raises the intriguing question of how is AMPylated Rab1 targeted and inactivated by LepB in vivo. We hypothesized the existence of a de-AMPylase, although such an enzyme has not yet been described in a pathogenic systems.

Whole cell lysate from *L. pneumophila* efficiently removed radiolabeled [a^{32}P] AMP from AMPylated Rab1. We were able to identify that SidD was the only Rab1-specific de-AMPylase encoded by *L. pneumophila*. In support of this finding, whole cell lysate generated from Δ*sidD* mutant strains showed no de-AMPylation activity toward Rab1. We found that SidD activity differs from that of the GS-ATase (glutamine synthetase adenylyl transferase), the only other de-AMPylase described to date, in that it is independent of the presence of phosphate and the byproduct of de-AMPylation is AMP and not ADP, even in the presence of phosphate. In addition, we show that Rab1 can be repeatedly AMPylated and de-AMPylated by SidM and SidD, respectively.

L. pneumophila Δ*sidD* mutant strains were defective for the timely removal of Rab1 from the LCV, suggesting that SidD is crucial for Rab1 inactivation and removal from the LCV. In vitro, purified SidD allowed de-AMPylated Rab1 to be inactivated by LepB. Collectively, our results point to SidD as the missing link between the processes of early Rab1 activation and subsequent inactivation later during host cell infection. Notably, SidD is the first de-AMPylase to be identified in a pathogenic system.

2 Identification and Characterization of Mammalian EFR3 Proteins as Phosphatidylinositol 4-Kinase Interacting Partners

Naveen Bojjireddy and Tamas Balla

Phosphoinositides represent a minor fraction of total membrane lipids that are critically important for various cellular functions. They are highly dynamic molecules formed by rapid phosphorylation of their inositol ring by specific kinases. Phosphatidylinositol 4-kinase III alpha (PI4KIII alpha) was recently described as an essential host factor in Hepatitis C (HCV) viral replication but little is known about the regulation of this enzyme. Recent studies in yeast showed that Efr3p interacts with and regulates the yeast orthologue of PI4KIII alpha. EFR3 proteins are highly conserved and the Drosophila homolog, called rolling blackout (rbo) was reported to be critical in phototransduction in the eye with a mutant showing a defect in phosphoinositide hydrolysis. Here we cloned EFR3A and 3B, two isoforms present in humans and mouse. GFP tagged EFR3A and EFR3B localized to the plasma membrane due to palmitoylation at the N-terminus of both EFR3A and 3B. Mutations of the conserved cystine residues, the sites of palmitoylation, abrogated EFR3 localization to plasma membrane. Immunoprecipitation studies with HA-tagged EFR3A and 3B revealed their association with PI4KIII a, but not PI4KIII beta. We are currently investigating the consequences of RNAi-mediated knockdown of EFR3A and 3B in PLC activation and signaling in agonist-stimulated cells.

3 Characterizing the effect of long-range basepairing interactions on translational control of the unfolded protein response transcription factor Hac1

Cheryl G. Bolinger and Thomas E. Dever

Control of eukaryotic gene expression at the level of translation allows for a rapid response to intracellular and environmental stimuli. A classic example is the unfolded protein response (UPR). When the folding capacity of the ER is exceeded, the transmembrane kinase/endonuclease IRE1 triggers a signaling cascade to turn on expression of genes encoding protein-folding catalysts to restore cellular homeostasis. Central to UPR activation in yeast is the transcription factor HAC1p, whose mRNA is an IRE1 substrate. During normal growth conditions, HAC1 mRNA is constitutively expressed but protein levels are undetectable. It has been proposed that HAC1p expression is tightly regulated by a basepairing interaction between 21 complementary nucleotides present in the mRNA 5' UTR and intron. The implicit mechanism is that secondary structure created by UTR/intron interaction blocks scanning ribosomes, and that this translational block is alleviated by IRE1-mediated removal of the HAC1 intron through a non-conventional splicing reaction.

4 SidD, a novel deAMPlyase from *Legionella pneumophila*

Yang Chen

Legionella pneumophila is a Gram-negative bacterium that can cause Legionnaires' disease, a severe pneumonia in humans. Following infection, the pathogen hijacks the intracellular vesicle transport pathway and establishes a camouflaged compartment resembling host cell rough ER for its replication. The bacterium interferes with the early secretory route by several mechanisms, one of which involves exploiting the activity of the small GTPase Rab1, the key regulator of the host cell secretory pathway. The bacterial protein SidM modifies Rab1 through the covalent addition of adenosine monophosphate (AMP), a process known as AMPylation. Overproduction of SidM in tissue culture cells was shown to cause cytotoxicity, an effect that has been attributed to SidM's AMPylation activity. Here we characterized another effector protein SidD from *L. pneumophila* that catalyzed the removal of AMP from Rab1, a novel activity named de-AMPylation. Using SidD variants with N- or C-terminal truncations in vitro assay we mapped the de-AMPylation activity region to the central part of SidD. COS1 cells producing fluorescently labeled SidD showed partial overlapping localization of SidD to the Golgi, consistent with the primary localization of its target Rab1. Fragments containing the C-terminus of SidD showed similar localization as full-length SidD. In contrast, no colocalization with specific cellular compartment was detected with SidD fragments lacking the C-terminus, indicating a role of the C-terminus for SidD localization. Cytotoxicity studies revealed that simultaneous production of SidD led to a significant reduction of cytotoxicity in cells producing SidM. Moreover, only the SidD fragment with both the de-AMPylation activity and the C-terminus localization signal is sufficient to rescue the cytotoxicity. Any fragment lacking the C-terminus showed no attenuation of the cytotoxicity due to the mislocalization of the protein. The de-AMPylation reaction catalyzed by SidD was specific for Rab1 because SidD failed to reduce the cytotoxicity caused by AMPylation of Rho GTPases by VopS, an effector protein encoded by Vibrio parahaemolyticus.

In this study, we analyzed a new effector protein SidD which exhibited an antagonistic activity of another effector protein SidM towards the same substrate. Our studies added another piece of information on how *L. pneumophila* effectors could modulate the host cell small GTPase Rab1.

5 piY RNA: Discovery, retinal enrichment and matrin 3 ligand properties

Samuel Clokie, Pierre Lau, David Klein

Small RNAs are critical elements in cellular regulation, functioning predominantly to reduce the levels of transcript and/or protein levels. Here we report the discovery of a 27nt RNA termed piY RNA, with features somewhat similar to those of piRNA, in that it is longer than the typical ~23 nt miRNA. piY RNA was identified during a cloning-based survey of pineal microRNAs and later found by Northern Blot to be expressed in the retina at levels that are greater than 20-fold higher than those in 14 other tissues examined. A proteomic search for binding partners for piY RNA found that matrin 3 is the predominant binding partner. Matrin 3 is a nuclear matrix protein implicated in several nuclear processes including transcription and editing. Other piY RNA-associated proteins that were identified include hnrpul1 (heterogeneous nuclear ribonucleoprotein U-like protein) and NCOA5 (nuclear receptor coactivator 5). Mutation of the wild type piY RNA sequence reduced or compleltey abrogated the interaction with matrin 3 – identifying critical bases required for the interaction. Matrin 3 (95 kDa) has two ~10 kDa RNA recognition motifs (RRMs), neither of which alone significantly binds piY RNA. However a ~22 kDa sequence containing both RRMs exhibits high affinity for piY RNA; indicating both are required for binding. Studies using full length recombinant matrin 3 and endogenous matrin 3 indicate that elements outside of the RRM region contribute to binding specificity. Additionally, phosphorylation by cyclic AMP dependent protein kinase enhances piY RNA to matrin 3. piY RNA was also found to inhibit translation in a model system. These results are of interest because they raise the possibility that the piY RNA/matrin 3 interaction might alter the biology of the retina and other tissues.

6 A Quantitative Protein Biomarker Time Course Study of Niemann-Pick Disease, type C

Stephanie M. Cologna, Xiaosheng Jiang, Peter S. Backlund, Christopher A. Wassif, Alfred L. Yergey and Forbes D. Porter

Niemann-Pick Disease, type C (NPC) is a fatal, neurodegenerative, genetic disorder in which the etiology is the accumulation of cholesterol and other lipids in the late endosomes and lysosomes. A cascade of pathological events occurs as a result of lipid accumulation which may serve as therapeutic targets for NPC. The phenotypic spectrum of NPC is broad and clinical symptoms tend to vary with respect to onset and increase in severity over time making treatment challenging. In this study we sought to gain further biochemical insight and identify protein markers that could serve as targets in therapeutic trials.

We used two-dimensional gel electrophoresis and mass spectrometry to identify differentially expressed cerebellar proteins in female $Npc1^{-/-}$ mutant mice compared to controls at 1, 3, & 5 weeks of age. Based on gel imaging, 77 spots were over-expressed ($R>1.5$, $p<0.05$) and 32 were under-expressed ($R<0.67$, $p<0.05$) over the time course study. The breakdown includes 18 under-expressed and 46 over-expressed proteins at one week of age, 6 proteins under-expressed versus 17 being over-expressed at week 3 and at week five, 8 protein spots under-expressed versus 14 being over-expressed in the mutant relative to control. To date, 59 unique proteins have been identified and we have chosen to focus on a small number of candidates to validate and further investigate. These include, fatty acid binding protein 3 (FABP3), and fatty acid binding protein 7 (FABP7).

Fatty acid binding proteins are responsible for the transport and metabolism of fatty acids. Specifically, FABP3 has been shown to bind omega-6 fatty acids while FABP7 preferentially binds omega-3 fatty acids. Both forms of fatty acid binding protein have been identified in the cerebellum and increased expression of both types has been correlated with brain injury. Interestingly, FABP3 is only up-regulated in the week five mutant mouse whereas FABP7 appears to be over-expressed in both weeks three and five of the NPC mutant mouse. To relate our findings from the mouse model to humans, increased FABP3 expression levels were validated using ELISA in cerebrospinal fluid from NPC patients relative to controls. Additional experiments are currently underway to further understand the biological significance of these two proteins in NPC.

7 Variable Experience with Adrenalectomy as Treatment for Congenital Adrenal Hyperplasia (CAH)

Melissa Crocker, MD, Carol Van Ryzin, CPNP, and Deborah Merke, MD

Background: Patients with CAH may undergo bilateral adrenalectomy in cases where adrenal androgens have been difficult to control medically.

Case 1: A 22-year-old female was noted to have ambiguous genitalia at two years of age and was diagnosed with CAH due to 21-hydroxylase deficiency. During adolescence, the patient had persistently high androgens and developed secondary amenorrhea from age 15 onward. Despite adding flutamide to her glucocorticoid and mineralocorticoid regimen, the patient continued to have amenorrhea and had hyperplastic adrenal glands on imaging. At 21 years of age she desired fertility and underwent laparoscopic bilateral adrenalectomy. Following the surgery, she had regular menses and no biochemical hyperandrogenism. She has conceived two pregnancies naturally without complications, 11 and 26 months following adrenalectomy.

Case 2: A 31-year-old female was born with Prader 4 genitalia and subsequently was diagnosed with 21-hydroxylase deficiency. Despite standard doses of suppressive glucocorticoid therapy, she developed significant virilization. At 16 years of age imaging revealed bilateral adrenal hyperplasia, and adrenal, ovarian, and renal venous sampling excluded other sources of androgens. She underwent bilateral adrenalectomy via laparotomy with initial improvements in her hyperandrogen symptoms. However, over the next 10 years with intermittent compliance of medications, her hyperandrogenism returned. Following a Liddle's dexamethasone suppression test (0.5 mg q6hr for 8 doses), her 17-OH-progesterone fell from 1750 ng/dL to <40, androstenedione from 494 ng/dL to 21, and testosterone from 339 ng/dL to 15. We suspect that a chronic course of noncompliance may have aggravated the production of ACTH, activating adrenal rest tissue. Imaging studies have not found the source of the adrenal rest tissue.

Conclusion: Following adrenalectomy, ectopic adrenal rest tissue may continue to produce androgens in CAH patients under the control of ACTH, leading to recurrence of virilization. Successful treatment of CAH with adrenalectomy may depend on the presence of adrenal rest tissue; however, identification and localization of adrenal rest tissue is difficult, especially in females. Future studies could use 18F-FDG-PET/CTs before and after cosyntropin stimulation testing to identify this tissue.

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8 Investigating the Mechanism and Pathophysiology of ISCU Myopathy

Daniel Crooks, Ronald Haller, Cathy Wu, Tracey Rouault

Recently, a hereditary myopathy (ISCU Myopathy; myopathy with deficiency of succinate dehydrogenase and aconitase) was found to be caused by a point mutation in the gene encoding the iron-sulfur cluster scaffold protein ISCU. This disease mutation confers a splicing defect that results in the inclusion of an additional intron sequence in the protein coding region of the ISCU mRNA, resulting in a premature stop codon and greatly depleted levels of ISCU protein in affected muscle tissue. Many questions remain about the pathophysiology of ISCU Myopathy, including the basis for tissue specificity of the disease, and a detailed molecular mechanism linking the aberrant ISCU mRNA transcript(s) to the greatly decreased protein levels observed in patient muscle biopsies. In order to address these questions on a detailed level, we obtained primary myoblasts derived from ISCU Myopathy patients. Here we show that ISCU protein levels are depleted in differentiating patient myotube cultures, leading to decreased activity and protein abundance of [4Fe-4S]-containing cytosolic and mitochondrial aconitases, and demonstrating a biochemical phenotype similar to that of affected patient tissues. Interestingly, the abundance of the [2Fe-2S]-containing ferrochelatase was also decreased in the patient cultures. Currently, we are using this myotube culture system to further investigate the pathophysiology of diseases caused by deficiencies in iron sulfur cluster biosynthesis. Furthermore, we aim to elucidate new pathways involved in mitochondrial iron homeostasis and regulation.

9 Protective bacterial toxin TisB produces well-defined anion-selective pores in planar lipid bilayers

Philip Gurnev, Ron Ortenberg, Kim Lewis, and Sergey Bezrukov

Recently identified small bacterial peptide TisB is a component of a toxin/antitoxin system. TisB toxin induces formation of drug-tolerant persister cells in response to DNAdamage. We have found that TisB forms well-defined ion-conductive pores in planar lipid bilayers. Using high-resolution conductance recordings with membranes of varying lipid compositions, we show that bath solution concentrations of TisB higher than 10 μM induce multilevel conductive states, which resemble pore formation by the well-known channel-former antibiotic alamethicin. In 1 M KCl TisB-induced pores usually first appear as stable conductive ohmic states (0.5, 1.5, and 2.6 nS), and, as time progresses, tend to produce various higher conductive states. The transition to these states is also favored by application of higher positive or negative transmembrane voltages. Both low and high conductive states possess close anion selectivity (~80 % anion current, measured in 1M/0.1M KCl salt gradient). Probing TisB pores in their lowest conductive states with differently-sized polyethylene glycols (PEGs) shows only minute polymer partitioning even for the smallest PEGs of 200 and 300 molecular weight. This finding implies that the lowest conductive states are characterized by relatively small diameter of the aqueous pores. TisB apparently creates a dormant state in persister cells by decreasing the proton motive force across their membranes and reducing ATP production, which leads to antibiotic tolerance.

10 Protein Targeting and Degradation Pathways are Coupled for Elimination of Mislocalized Proteins

Tara Hessa, Ajay Sharma, Malaiyalam Mariappan, Heather D. Eshleman, Erik Gutierrez, and Ramanujan S. Hegde

A substantial proportion of the genome encodes membrane proteins that are delivered to the endoplasmic reticulum by dedicated targeting pathways. Membrane proteins that fail targeting must be rapidly degraded to avoid aggregation and disruption of cytosolic protein homeostasis. The mechanisms of mislocalized protein (MLP) degradation are unknown. Here, we reconstitute MLP degradation in vitro to identify factors involved in this pathway. We find that nascent membrane proteins tethered to ribosomes are not substrates for ubiquitination unless they are released into the cytosol. Their inappropriate release results in capture by the Bag6 complex, a recently identified ribosome-associating chaperone. Bag6 complex capture depends on unprocessed or non-inserted hydrophobic domains that distinguish MLPs from potential cytosolic proteins. A subset of these Bag6 clients is transferred to TRC40 for membrane insertion, while the remainder are rapidly ubiquitinated. Depletion of the Bag6 complex impairs efficient ubiquitination selectively of MLPs. Thus, by its presence on ribosomes synthesizing nascent membrane proteins, the Bag6 complex links targeting and ubiquitination pathways. We propose that such coupling permits fast-tracking of MLPs for degradation without futile engagement of cytosolic folding machinery.

11 A novel mechanism for regulation of ribosome biogenesis through the oncogene B23/nucleophosmin and the GTPase Ran.

Yonggang Wang, **Ming-Ta Lee**, **Shaofei Zhang**, Peter Backlund, Alfred Yergey and Mary Dasso

Alterations in nucleolar morphology are commonly observed in cancers, reflecting both increased ribosome biogenesis and changes cell cycle regulatory mechanisms. Ribosome biogenesis is a complex process that constitutes the largest metabolic expense of eukaryotic cells: In budding yeast, it involves around 80 ribosomal proteins and four ribosomal RNAs (rRNAs), as well as over 200 non-ribosomal assembly factors. Many efforts at understanding nucleolar transformations found in cancer cells focus on B23/nucleophosmin (B23/NPM1), a nucleolar protein that is essential for ribosome biogenesis and that is frequently mutated in hematopoietic malignancies. We have previously demonstrated that B23/NPM1 binds to SENP3 and controls its stability. SENP3 is a vertebrate SUMO protease, which reverses the conjugation of cellular target proteins to small ubiquitin-related modifiers (SUMOs). The covalent conjugation of cellular proteins to SUMOs occurs in a manner that is reminiscent of ubiquitination. The SUMO pathway plays an essential role in ribosome biogenesis in both yeast and vertebrates, and loss of either SENP3 or B23/NPM1 blocks the assembly pathway at a late step, 32S to 28S pre-rRNA processing. Here, we identified SENP3-associated proteins within *Xenopus* egg extracts (XEEs), and found that SENP3 tightly binds three proteins of undefined function: PELP1, Tex10, and WDR18. Like SENP3, each of these proteins localized within nucleoli and was essential for 32S to 28S pre-rRNA processing. Remarkably, sequence analysis indicated that they are previously unrecognized homologues of *S. cerevisiae* Rix1 complex members (Rix1p, Ipi1p and Ipi3p), which are essential for late stage pre-rRNA processing and export of 60S ribosomal particles from the nucleus in yeast. Both vertebrate Rix1 complex members and SENP3required B23/NPM1 for binding to 60S ribosomal particles in XEEs. Notably, this binding also required high levels of Ran-GTP, a small nuclear GTPase that controls the direction of nuclear trafficking. Under low Ran-GTP conditions, B23/NPM1 was excluded from the complex and the nuclear import receptor RanBP5 bound instead. Taken together, our findings demonstrate striking conservation between the ribosome biogenesis pathways in yeast and vertebrates, and suggest a novel mechanism through which the vertebrate pathway may be regulated by Ran. Importantly, they also provide a clear molecular role for a critical oncogene, B23/NPM1, in the ribosome assembly pathway.

12 Histone chaperone DAXX is a polySUMO binding protein

Lyanguzova M., Arnaoutov A., Dasso M.

SUMO proteins are a family of Ubiquitin-like modifiers that become covalently attached (conjugated) to many other cellular proteins. SUMO conjugation targets play essential roles in many processes, including gene expression, cell cycle progression, synthesis and repair of DNA, response to extracellular stimuli and nucleocytoplasmic transport. SUMO proteins have been implicated in many human diseases, including cancers, Alzheimers disease, Huntingtons disease, Parkinsons disease and type I diabetes. Individual targets can be conjugated to a single SUMO, or SUMO's can form chains on their target protein; these distinct conjugation patterns confer alternative fates because they promote different sets of protein-protein interactions. While the recognition of SUMO monomers has been extensively studied, recognition of polySUMO chains has not been well characterized. We've identified polySUMO binding proteins in Xenopus laevis egg extracts through pull-down assays, using recombinant single SUMO proteins or tandem SUMO polypeptides as bait. SDS-PAGE analysis showed a clear difference between monomer- and chain-binding proteins. Mass-spectroscopy analysis (MS) of these proteins gave a list of around 80 proteins that bind SUMO chain, 50 of which were not found in the mono-SUMO samples, indicating that they are likely to be polySUMO-specific. We are exploring the function of a number of SUMO chain-specific proteins, including the protein DAXX (Death domain-associated protein 6). Immunoblotting data confirmed that DAXX binds polySUMO chains, but not monomers. DAXX acts as a chaperone for replication-independent loading of histone H3.3 (H3.3) on pericentromeric and telomeric chromatin as well as on the transcription factors binding sites. We hypothesize that DAXX interacts with chromatin and uploads H3.3 on at least some regions of the chromosome in SUMO-dependent manner. We are currently utilizing chromatin immunoprecipitation followed by massively parallel sequencing (ChIP-seq)to compare the distribution of wild type DAXX protein to mutants whose SUMO binding domains have been disrupted. We expect that these experiments will provide insight into the role of SUMOylation in the organization of chromosomes, and allow molecular characterization of mechanisms targeting H3.3 distribution.

13 Reconstitution of Tail-Anchored Membrane Protein Insertion from Purified Components

Malaiyalam Mariappan, Elia Bove, Agnieszka Mateja, Robert J. Keenan, and Ramanujan S. Hegde

Tail-anchored (TA) membrane proteins encompass a widely expressed class of factors with diverse biological functions. The single transmembrane domain (TMD) of TA proteins is chaperoned by cytosolic targeting factors that deliver it to membrane receptors for insertion into the lipid bilayer. While a basic framework for TA protein recognition is now emerging, the decisive targeting and membrane insertion steps remain poorly understood. Here, we reconstitute TA membrane protein insertion with recombinant components. A targeting complex of TA protein bound by the chaperone ATPase Get3 was delivered to an integral membrane receptor complex of Get1 and Get2. At the membrane, Get1/2 orients Get3 and spatially regulates its ATPase-dependent TA protein release to favour membrane insertion and preclude aggregation. ATP then primes the substrate-free Get1/2/3 complex for another round of TA protein insertion. This work defines the minimal components of a conserved membrane insertion pathway, delineates each step in the insertion cycle, and paves the way for detailed mechanistic analysis.

14 La-related protein 4 binds poly(A), interacts with the poly(A)-binding protein MLLE domain via a variant PAM2w motif, and can promote mRNA stability.

Mattijssen S, Yang R, Gaidamakov SA, Xie J, Lee J, Martino L, Kozlov G, Crawford AK, Russo AN, Conte MR, Gehring K, Maraia RJ.

The conserved RNA binding protein La recognizes UUU-3'OH on its small nuclear RNA ligands and stabilizes them against 3'-end-mediated decay. We report that newly described La-related protein 4 (LARP4) is a factor that can bind poly(A) RNA and interact with poly(A) binding protein (PABP). Yeast two-hybrid analysis and reciprocal immunoprecipitations (IPs) from HeLa cells revealed that LARP4 interacts with RACK1, a 40S ribosome- and mRNA-associated protein. LARP4 cosediments with 40S ribosome subunits and polyribosomes, and its knockdown decreases translation. Mutagenesis of the RNA binding or PABP interaction motifs decrease LARP4 association with polysomes. Several translation and mRNA metabolism-related proteins use a PAM2 sequence containing a critical invariant phenylalanine to make direct contact with the MLLE domain of PABP, and their competition for the MLLE is thought to regulate mRNA homeostasis. Unlike all ~150 previously analyzed PAM2 sequences, LARP4 contains a variant PAM2 (PAM2w) with tryptophan in place of the phenylalanine. Binding and nuclear magnetic resonance (NMR) studies have shown that a peptide representing LARP4 PAM2w interacts with the MLLE of PABP within the affinity range measured for other PAM2 motif peptides. A cocrystal of PABC bound to LARP4 PAM2w shows tryptophan in the pocket in PABC-MLLE otherwise occupied by phenylalanine. We present evidence that LARP4 expression stimulates luciferase reporter activity by promoting mRNA stability, as shown by mRNA decay analysis of luciferase and cellular mRNAs. We propose that LARP4 activity is integrated with other PAM2 protein activities by PABP as part of mRNA homeostasis.

15 Identification of key structural features of a poxviral homolog of anaphase promoting complex subunit 11

Min Mo, Stephen B. Fleming, and Andrew A. Mercer

The catalytic core of the multi-subunit ubiquitin ligase anaphase promoting complex (APC), a key cell cycle regulator, is composed of subunits 2 (APC2) and 11 (APC11). APC11 is a RING-H2 protein that binds to the cullin homology domain (CHD) of APC2.

The parapoxvirus, Orf virus, encodes a RING-H2 protein, PACR (Poxvirus anaphase complex regulator), with clear sequence similarities to APC11. Here we show that B5L binds to APC2 in a manner similar to APC11. However, unlike APC11, PACR failed to demonstrate ubiquitin ligase activity in an *in vitro* ubiquitination assay. Tertiary structure modelling indicates that the lack of ubiquitin ligase activity results from a distinctive sequence variation within the RING-H2 domain of PACR and its poxviralhomologues. When this region of PACR (amino acids 59-67) was replaced with corresponding region of APC11 (amino acids 61-74), the mutated PACR acquired ubiquitin ligase activity. On the other hand, APC11 in which the domain was replaced with that of PACR lost ubiquitin ligase activity. In order to confirm the predicted structural role of this domain, attempts to purify and crystallize PACR and APC11 are currently underway.

Our data suggest that PACR is an inhibitor of APC. However, the mechanism of PACR inhibition of APC remains to be elucidated. Does PACR inhibit APC by directly competing with endogenous APC11 binding to APC2 or does it disrupt the integrity of APC by other means? In order to investigate these possibilities, *in vitro* binding assays of the interaction between PACR and purified APC subunits are being conducted.

16 Blast wave induced membrane injury

Shay M. Rappaport, Alexander Berezhkovskii, Rea Ravin, Sergey Bezrukov and Joshua Zimmerberg

The number of soldiers and citizens exposed to explosive detonation increases every year. Among other sources of blast injuries the most unclear yet not least severe is the so called “primary” blast wave. In this case the injury is caused directly by the shock wave itself and not by any penetrating fragments or other body and hard object interaction. While it is relatively simple to explain how a high amplitude pressure wave can cause a damage to gas filled structures (i.e., lungs, middle ear, etc.) it is not clear, although broadly investigated, how it causes a traumatic brain injury (TBI). One suggestion is that the injury is induced at the molecular level. A theoretical mechanism of a blast shock wave induced membrane injury is described. According to the model the rapid drop of pressure from high to a milliseconds-long period of negative pressure produces a cavitation between membrane leaflets which leads to membrane stress, poration or even rupture. We suggest that this mechanism may be one of the causes of traumatic brain injury (TBI) due to the blast wave.

17

Catalysis of lysine 48-specific ubiquitin chain assembly by residues in E2 and ubiquitin.

Monica C. Rodrigo-Brenni, Scott A. Foster and David O. Morgan

Protein ubiquitination is catalyzed by ubiquitin-conjugating enzymes (E2s) in collaboration with ubiquitin-protein ligases (E3s). This process depends on nucleophilic attack by a substrate lysine on a thioester bond linking the C terminus of ubiquitin to a cysteine in the E2 active site. Different E2 family members display specificity for lysines in distinct contexts. We addressed the mechanistic basis for this lysine selectivity in Ubc1, an E2 that catalyzes the ubiquitination of lysine 48 (K48) in ubiquitin, leading to the formation of K48-linked polyubiquitin chains. We identified a cluster of polar residues near the Ubc1 active site, as well as a residue in ubiquitin itself, that are required for catalysis of K48-specific ubiquitin ligation, but not for general activity toward other lysines. Our results suggest that the active site of Ubc1, as well as the surface of ubiquitin, contains specificity determinants that channel specific lysines to the central residues involved directly in catalysis. (1) (2)

(1) This research was done as part of my PhD work in the laboratory of David Morgan at UCSF and it is published (Mol Cell. 2010 Aug 27;39(4):548-59).

(2) Currently I am a Postdoctoral Fellow in the laboratory of Ramanujan Hegde.

18

Age-related changes in DNA methylation in human peripheral blood monocytes

Paraskevi Salpea, Valya R. Russanova, Tazuko Hirai, Bruce H. Howard

It has been reported that epigenetic changes, including changes in DNA methylation, occur during development and aging. However, much remains to be learned on a whole genome-scale regarding age-associated epigenome dynamics. Our aim is to reveal key genomic regions, with distinct structure and sequence characteristics, that appear to be susceptible to age- related deregulation of epigenome methylation patterns. We investigated, for the first time, the human methylome pattern on a whole genome basis in monocytes and in vitro differentiated dendritic cells from newborns and adults. For this purpose, we performed Methylated DNA Immunoprecipitation (MeDIP) followed by Next generation Sequencing (MeDIP-Seq). To minimize differences in methylome data due to individual variation, we pooled DNA samples for each age group in all the experiments. The results were adjusted to an input DNA for the precipitation and further normalized relative to the CpG content. Two different kinds of genomic regions that alter their methylation pattern with age emerged. The first consist of clustered/ array genes and genes associated with multiply aligned sequences such as PCDHG, FAM90A and HRNR. The second group contained genes rich in CG (>55%), with regions of CpG islands (>200bp), islets (<200bp) and “micro-islands” (<100bp), such as FZD1, FZD7 and FGF17. Single copy areas rich in CG such as CpG islands, islets and “micro-islands” and multiply aligned sequences (Repeat Masker-excluded) around a gene or within a gene cluster are distinct sequence structures that appeared to be susceptible to DNA methylation changes with age.

19 Molecular Mechanisms of Transcription Regulation of the Imprinted Genes *H19* and *Igf2*

Megan Sampley and Karl Pfeifer

Genetic imprinting is an epigenetic mode of gene expression regulation that results in monoallelic gene transcription depending upon parental inheritance of the chromosome. Imprinted genes are often found closely positioned in clusters where several genes are coordinately regulated by shared *cis* elements in the neighboring DNA sequence. A well-characterized imprinted gene cluster occurs at the distal end of mouse chromosome 7, which harbors the closely positioned *Igf2* (*Insulin like growth factor-2*)and *H19* genes. *H19* and *Igf2* are monoallelically expressed from the maternal and paternal alleles, respectively, in a coordinated mode of regulation by several *cis* regulatory elements spread over the approximately 150kb *Igf2/H19* gene locus. Imprinting in this region is maintained by several differentially methylated regions (DMR), one of which is an imprinting control region (ICR) located between the *H19* and *Igf2* genes. The ICR is sufficient to mark the parent-of-origin state of the allele and direct monoallelic expression. In addition to allele-specific transcription regulation by the ICR, the tissue-specific expression of *H19* and *Igf2* is controlled by two tissue-specific enhancers that lie downstream of *H19*. The ICR functions insulator element that controls gene expression by physically interacting with these downstream enhancers and blocking their access to either the *H19* or *Igf2* promoters. These physical interactions between the ICR, enhancers and promoters are regulated by both the methylation status of the ICR as well as the binding of the insulator protein CTCF (CCCTC-binding factor) at the ICR. These physical interactions create DNA loop structures that coordinate gene expression in both an allele-specific and tissue-specific manner. While the effects of many *cis* elements in the H19/Igf2 locus have been well-characterized through genetic studies, relatively little information is known about *trans*-acting regulatory proteins that bind to these sites and how they regulate chromatin structure. Our current studies are aimed at characterizing the synergistic effects of both *cis* and *trans* factors on overall chromatin structure, including histone modifications and DNA loop formation, and how these processes regulate gene expression. Of particular interest, our group is interested in how *cis*-elements in the *Igf2/H19* region regulate histone modifications along the entire locus and how transcription factors, histone-modifying enzyme complexes and additional transcription machinery direct these processes. Such studies will be beneficial in further elucidating the molecular mechanisms of imprinting.

20 Mechanistic basis of host-cell remodeling by the HIV-1 protein Vpu

Zai-Rong Zhang and Ramanujan S. Hegde

A fundamental feature of all viruses is their exploitation of host cell machinery to facilitate their infection, intracellular residence, and replication. A major mechanism of this exploitation is to remodel the host cell by altering its protein composition to favor the viral life cycle. Many viral genomes therefore encode proteins that selectively and potently target certain host cell proteins for degradation. HIV-1 also uses this general strategy to selectively downregulate CD4, an essential receptor for HIV-1 entry into cells. This downregulation is important for preventing superinfection, maximizing virion production, and achieving full infectivity of the released virons. Stable CD4 downregulation is dependent on the HIV protein Vpu, a small membrane protein of only 81 amino acids. Previously studies have established that Vpu interacts with newly synthesized CD4 at the endoplasmic reticulum to mediate CD4 ubiquitination. Ubiquitinated CD4 is then extracted from the ER membrane and degraded by the proteasome in the cytosol. The ubiquitin ligase used by Vpu to mediate CD4 ubiquitination is thought to be the SCFb-TrCP. While this basic pathway has been defined on the basis of cell culture studies, the molecular mechanisms that mediate this ubiquitination or the downstream degradation are not well understood. In order to dissect the detailed mechanism by which Vpu induces CD4 degradation on the endoplasmic reticulum membrane of the host cell, we reconstituted the Vpu induced CD4 ubiquitination in vitro, using recombinant Vpu and SCFb-TrCP complex, in vitro translated and radiolabeled CD4 and the synthetic liposome. We have been able to incorporate the membrane protein Vpu and CD4 into the liposome and their interaction was determined by co-IP. Furthermore, by incubating the liposome with recombinant SCFb-TrCP complex, the CD4 get ubiquitinated significantly in a phosphorylated Vpu dependent manner. In addition, we purified rough microsome containing Vpu from Vpu stably expressed cell line. We found that in vitro translated CD4 on this microsome can be ubiquitinated probably by the SCFb-TrCP complex pre-associated with the microsome membrane. The ubiquitinated CD4 can be dislocated from microsome in a cytosol dependent manner. Combining these in vitro systems, future studies are expected to provide mechanistic insight into how Vpu escapes from direct ubiquitination by SCF complex and which factor channeled the extraction of CD4 from ER membrane to cytosol.

21 Intracellular trafficking and distribution of VEGF165 in living cells suggests a non-conventional secretory pathway.

Maria L. Guzmán-Hernández, Gael Potter, Yeun J. Kim,Jozsef Z. Kiss and Tamas Balla

Vascular endothelial growth factor (VEGF) is a major regulator of physiological and pathological angiogenesis. The VEGF gene is spliced to yield four mature isoforms of 121, 165, 189, and 206 residues. While the biological roles of VEGF isoforms have been extensively studied, as have been the means to interfere with VEGF actions in order to prevent cancer growth, the intracellular trafficking and mode of secretion of VEGF remain largely unknown. In this study we tried to address this important question by creating VEGF165 fused with GFP (VEGF165-GFP) and following its trafficking in living COS-7 cells by confocal microscopy. In parallel, we set up an assay to measure the release of VEGF165-GFP into the media using trichloroacetic acid precipitation and quantifying GFP by Western blot analysis. First, we determined that VEGF165-GFP forms dimers and gets glycosylated similarly to wild-type VEGF165 and induces vascularization when transduced into mice with a lentiviral delivery system. Confocal analysis of COS-7 cells or HUVEC cells revealed that VEGF165-GFP initially followed the endoplasmic reticulum-Golgi pathway but, intriguingly, its post-Golgi trafficking did not conform the classical constitutive secretory pathway: VEGF165-GFP secretion still occurred at 19 °C, while constitutive secretion is known to cease below 22 °C. Moreover VEGF165-GFP remained associated with discrete patches at the outer surface of the plasma membrane that is not observed with other constitutively secreted proteins. Immunostaining of cells without permeabilization confirmed that expressed untagged VEGF165 also was associated with the outer cell surface. The secretion of VEGF165 increased after elevation of intracellular Ca^{2+} or activation of PKC. Importantly, mutation of the single N-glycosylation site in VEGF168-GFP or inhibition of glycosylation, both prevented secretion of the VEGF165-GFP protein. The secretion of endogenous cyclophilin B, which follows the classical secretory pathway, was used as a control. Our results clearly showed that the secretion of VEGF165 depends on glycosylation and follows a pathway with unique properties including regulation by Ca^{2+} and PKC. Taken together, our improved understanding of the exact biochemical steps involved in VEGF secretion could identify new strategies to prevent angiogenesis and hence fight cancer progression.

22 Regulation of PI4KIIa Retrograde Transport

Marko Jovic, Zsofia Szentpetery, Michelle Kean, Anne-Claude Gingras, Julie Brill, and Tamas Balla

Proper function of each cellular compartment relies on maintenance of the unique lipid composition of its membrane. In the Golgi, phosphatidylinositol 4-phosphate (PtdIns4*P*) is the key regulatory lipid that mediates sorting of proteins out of this compartment. Interestingly, while several PtdIns4*P* lipid kinases (PI4Ks) localize to the Golgi, a large fraction of PI4KIIa is also found on endosomes. Tight membrane association of PI4KIIa through a palmitoyl moiety implies that this enzyme must continuously cycle between endosomes and Golgi. The importance of PI4KIIa localization is underscored by its role in EGF receptor degradation, Wnt signaling and a late-onset neurodegeneration. Surprisingly, little is known about the molecular events determining the localization and functions of PI4KIIa in various cellular compartments. This study was designed to address the regulation of PI4KIIa transport between Golgi and endosomes.

To this aim we performed a proteomic screen of potential binding partners of PI4KIIa using tandem mass-spectrometry analysis of proteins that were co-immunoprecipitated (Co-IP) with PI4KIIa. One of the highest affinity partners was a vesicular fusion protein VAMP-3 that is known to facilitate fusion of endosome-originated membranes with the Golgi. This interaction was confirmed in Co-IP experiments, by performing a pull-down of either VAMP-3 or PI4KIIa followed by blotting against the other binding partner. Remarkably, expression of Tetanus toxin (TeNT), which cleaves VAMP-3 and inhibits one of the retrograde trafficking routes, resulted in accumulation of PI4KIIa on endosomes as determined by live-cell confocal microscopy. In a control rescue experiment, overexpression of TeNT together with mutant VAMP-3 resistant to TeNT recovered the Golgi localization of PI4KIIa. TeNT will, therefore, serve as a valuable method in our future kinetic studies where a photoactivatable variant of PI4KIIa will be used to assess the rate of its retrograde transport back to the Golgi in the presence of TeNT.

Taken together, these findings provided the first evidence of the potential mechanism regulating PI4KIIa retrograde transport, and revealed new approaches by which to study the role of PI4KIIa in cellular processes relevant to human health and disease.

23 Developmental synaptic NMDA receptor remodeling by Kv4.2 potassium channels *in vivo*

Eun Young Kim and Dax A. Hoffman

In the hippocampal CA1 pyramidal neurons, excitability is controlled by the voltage gated potassium channel subunit, Kv4.2. We found that blockage or deletion of Kv4.2 increased the frequency and amplitude of dendritic action potentials. Also, Kv4.2 expression in cultured neurons increased with days *in vitro* suggesting a link between Kv4.2 and synapse maturation. Therefore, Kv4.2 knock-out (KO) mice along with acute *in vivo* overexpression of Kv4.2 were used to test whether Kv4.2 expression level influences synaptic development in the hippocampus. Electrophysiology recordings in acute hippocampal slicesshowed that the NMDA/AMPA ratio and synaptic NR2B fraction were higher in both neonatal WTand adult Kv4.2 KO than in adult WT. Acute overexpression of Kv4.2 by injecting Sindbis virus carrying EGFP-tagged Kv4.2 into the hippocampus lowered the NMDA/AMPA ratio and NR2B fractionin infected neurons compared to uninfected neurons in both neonatal WT and adult Kv4.2 KO. These resultsindicate for the first time that developmental synaptic modifications depend on Kv4.2 expressionlevel. To see if Kv4.2 KO delays synaptic maturation, silent synapses were measured by a minimal stimulation protocol. In neonatal Kv4.2 and WT neurons, silent synapses contribute 50% of total synapses. Surprisingly, neurons in adult Kv4.2 KO also were found to contain a large number (55%) of silent synapseswhile only 2% of synapses were silent in adult WT. Importantly, overexpression of Kv4.2 in neonatal WT reduced the incidence of silent synapses (29%). These results indicate that Kv4.2 might have an important role during development and disruption of Kv4.2 expression will delay synaptic maturation by altering synaptic modification, which may underlie abnormal CNS development and neuropsychiatric disorders.

24 Activation of STIM1-Orai1 involves an intramolecular switching mechanism

Marek Korzeniowski and Tamas Balla

The stromal interaction molecule (STIM1) regulates Ca^{2+} entry through plasma membrane Orai1 channels in response to decreased endoplasmic reticulum luminal Ca^{2+} concentration. We identified an acidic motif within the STIM1 coiled-coil region that keeps its Ca^{2+} activation domain (CAD/SOAR)—a cytoplasmic region required for its activation of Orai1—inactive. The sequence of the STIM1 acidic motif shows substantial similarity to that of the C-terminal coiled-coil segment of Orai1, which is the postulated site of interaction with STIM1. Mutations within this acidic region render STIM1 constitutively active, whereas mutations within a short basic segment of CAD/SOAR prevent Orai1 activation. We propose that during STIM1 activation, the CAD/SOAR domain is released from an intramolecular clamp allowing the basic segment to activate Orai1 channels. This evolutionarily-conserved mechanism of STIM1 activation resembles the regulation of protein kinases by intramolecular silencing through pseudosubstrate binding.

25 A Novel Quality Control Pathway Targets Misfolded Prion Proteins from the Endoplasmic Reticulum for Lysosomal Degradation

Prasanna Satpute-Krishnan, Ramanujan S. Hegde, Jennifer Lippincott-Schwartz

When misfolded proteins accumulate in cells, they can disrupt cellular function leading to disease. In particular, misfolded *secretory* proteins are associated with a variety of diseases including liver diseases, diabetes and neurodegeneration. To avoid this, cells have evolved quality control systems that identify terminally misfolded proteins and target them for degradation. The best-characterized degradation pathway for secretory proteins, endoplasmic reticulum associated degradation (ERAD), takes place in the ER where secretory proteins are synthesized. However, not all misfolded secretory proteins are recognized by the ERAD system, which raises the question: "How and where are these misfolded secretory proteins processed?"

To investigate alternate degradation pathways of secretory proteins, I created a fluorescent protein-tagged, terminally misfolded variant of the prion protein that is refractory to ERAD, called PrPmisf. Using live-cell imaging and radioactive pulse-chase with inhibitory drugs, antibody uptake and co-immunoprecipitation techniques, I have discovered that at steady state, most PrPmisf is retained in the ER by calnexin while a fraction traffics to the plasma membrane (PM) before being rapidly targeted to lysosomes. Treatment with agents that perturb binding of calnexin to PrP^{misf} results in the synchronous export of PrPmisf out of the ER, through the Golgi, to the PM, where it is rapidly endocytosed to lysosomes. An endocytic inhibitor can trap the PrPmisfon the PM. In contrast, newly synthesized wild type PrP (PrPwt) is efficiently exported and stably localized to the PM. Upon examining differences between the post-translational modifications of PrPmisf and PrPwt, I found that unlike PrPwt, the N-linked glycosylations of PrPmisf do not mature after trafficking through the Golgi. Taken together, these results suggest that PrPmisf and PrPwt are handled differently at each step of the secretory pathway, culminating in the selective targeting of PrPmisf by an, as yet, uncharacterized PM-level quality control.

Currently, I am evoking the synchronous trafficking of PrPmisf to isolate PrPmisf with its interactorsat each step of its degradation pathway. In parallel, I am identifying features of PrPmisf that route it to this path by mutational analysis. Thus, I aim to reveal the mechanistic details of this novel quality control pathway, with potentially broad relevance for other disease-related proteins that escape ERAD.

26 Dependence of the Pacemaking on the Basal TRP Channel Activity in Spontaneously Firing Pituitary Cells

Marek Kucka, Karla Kretschmannova, Hana Zemkova, Melanija Tomic and Stanko S. Stojilkovic

All secretory anterior pituitary cells fire action potentials spontaneously because their resting membrane potential is positive to the potassium equilibrium potential, but the channels involved in the background permeability have not been identified. Replacement of bath sodium with large organic cations, but not blockade of voltage-gated sodium influx, led to an instantaneous hyperpolarization of cell membranes that was associated with a cessation of spontaneous firing in lactotrophs and immortalized GH_3 cells. When cells were clamped at -50 mV, which was close to the resting membrane potential in these cells, replacement of bath sodium with organic cations resulted in an outward-like current, reflecting an inhibition of the holding membrane current and indicating loss of a backgrounddepolarizing conductance. Replacement of bath sodium with organic cations also abolished voltage-gated calcium influx in these cells. Lithium substituted for sodium in electrical activity and prolactin secretion. Quantitative RT-PCR analysis revealed the high expression of mRNA transcripts for TRPC1 and lower expression of TRPC6 in both lactotrophs and GH_3 cells. TRPC3, TRPC4, and TRPC5 mRNA transcripts were also present in pituitary but not GH_3 cells. 2-APB and SKF-96365, blockers of TRPC channels, inhibited electrical activity and basal prolactin release in a concentration-dependent manner. Gadolinium inhibited firing of action potentials directly, by inhibiting voltagegated calcium channels, and indirectly, by partially inhibiting the background depolarizing current. FFA, an inhibitor of nonselective cation channels, also partially inhibited this current, causing cessation of electrical activity, calcium influx and prolactin release. These results indicate that basal activity of TRPC channels contributes to the background depolarizing conductance and firing of action potentials in lactotrophs and GH_3 cells.

27 A High-Throughput Screen for Molecules that Selectively Induce DNA Re-replication in Cancer Cells

Chrissie Y. Lee, Wenge Zhu, Ronald L. Johnson, Jennifer Wichterman, Ruili Huang, Melvin L. DePamphilis

In normal mammalian mitotic cells, the genome is duplicated only once per cycle. Once DNA replication has begun, multiple mechanisms exist to ensure that DNA replication is not reinitiated again until the previous cell cycle has been complete. Geminin is a protein unique to metazoa and is required for the licensing of DNA replication, ensuring that replication firing at the origins occurs only once per cell cycle. Suppression of geminin leads to DNA damage, stalled replication forks, and cell death. Recent work in our lab has shown that siRNAs against geminin will cause cells derived from cancers to undergo unscheduled DNA re-replication and subsequent apoptosis. Conversely, cell lines derived from normal tissues will only undergo DNA re-replication if both geminin and cyclin A are suppressed. Thus, cells derived from normal tissues have additional mechanisms to prevent DNA re-replication, providing a unique strategy of selectively targeting cancer cells that could be applied to the discovery of potential anticancer therapeutics. To this end, we developed a high-throughput assay that will monitor DNA replication in excess of four genomic equivalents, as well as index cell proliferation of both cancerous and noncancerous cell lines. This assay was initially validated by screening a library of 1280 bioactive molecules and proved more sensitive than current methods for detecting excess DNA replication. This screen identified known inducers of excess DNA replication, including inhibitors of microtubule dynamics and novel compounds that induced excess DNA replication in both normal and cancer cells. Promisingly, some of the compounds selected from the screen are currently used as chemotherapies, including taxol, vinblastine, and vincristine. Thus, this high-throughput screening assay has proven potential as a new approach to discovering compounds useful for investigating the regulation of genome duplication and for the treatment of cancer. Currently, we are in the process of using the principles of this assay to screen a larger library of over 340,000 small molecules of unknown functions. From this screen we hope to identify novel compounds to be used therapeutically that will be selective in killing cancer cells without the toxic side effects seen in the current chemotherapeutic agents.

28 AKAP79/150 impacts intrinsic excitability of hippocampal neurons through phospho-regulation of A-type K^+ channel trafficking

Lin Lin, Wei Sun, Faith Kung, Mark L. Dell'Acqua and Dax A. Hoffman

Kv4.2, as the primary a-subunit of rapidly inactivating, A-type voltage-gated K^+ (Kv) channels expressed in hippocampal CA1 pyramidal dendrites, plays a critical role in regulating their excitability. Activity-dependent trafficking of Kv4.2 relies on C-terminal protein kinase A (PKA) phosphorylation. A-kinase anchoring proteins (AKAPs) target PKA to glutamate receptor and ion channel complexes to allow for discrete, local signaling. As part of a previous study, we showed that AKAP79/150 interacts with Kv4.2 complexes and that the two proteins colocalize in hippocampal neurons. However, the nature and functional consequence of their interaction has not been previously explored. Here, we report that the C-terminal domain of Kv4.2 interacts with an internal region of AKAP79/150 that overlaps with its MAGUK binding domain. We also show that AKAP79/150-anchored PKA activity controls Kv4.2 surface expression in heterologous cells and hippocampal neurons. Consistent with these findings, disrupting PKA anchoring lead to a decrease in neuronal excitability while preventingdephosphorylation by the phosphatase calcineurin resulted in increased excitability. These results demonstrate that AKAP79/150 provides a platform for dynamic PKAregulation of Kv4.2 expression, fundamentally impacting neuronal excitability.

29 Transport independent control of histone demethylase KDM3B by Crm1 and the Ran GTPase.

Kara Lukasiewicz, Alexei Arnaoutov, Peter Backlund, Alfred Yergey, Mary Dasso

The Ran GTPase controls nucleocytoplasmic transport of cargo proteins and RNAs. GTP-bound Ran (RanGTP) binds to transport receptors and modulates their association with cargo. Export receptors bind cargo and RanGTP within nuclei. After export, GTP hydrolysis occurs, resulting in complex dissociation. Conversely, import receptors bind cargo in the cytosol. After import, they bind RanGTP and release their cargo. In some cases, import complex dissociation is further conditional upon events within the nucleus, such as the assembly of cargo into macromolecular complexes, suggesting that import receptors might control not only translocation through the nuclear pore but also the intranuclear targeting or function of cargos. We have examined whether the export receptor Crm1 might likewise regulate cargo in a manner that is unlinked to export.

Crm1 pulldowns coupled with mass spectrometry (MS) identified putative histone demethylase lysine (K) demethylase 3B (KDM3B) as a novel Crm1 cargo that it binds with high affinity in the presence of RanGTP. Both *in vitro* histone demethylase assays measuring histone 3 lysine 9 mono-, di- and tri-methylation (H3K9-1me, -2me, and -3me) by MS analysis and immunofluorescence (IF) co-staining in HeLa cells overexpressing KDM3B suggests that KDM3B demethylates H3K9-2me. IF staining showed that KDM3B and Crm1 co-localize within the nucleoplasm and fibrillar center of nucleolus, the site of ribosomal RNA (rRNA) gene transcription. We hypothesized that association with Crm1 and RanGTP regulate KDM3B's localization, sites of genomic binding or enzymatic function. Strikingly, treatment with leptomycin B (LMB), an inhibitor of Crm1 cargo binding, caused complete and rapid loss of KDM3B from the nucleolus, indicating that Crm1 is required for KDM3B nucleolar localization. Cells treated with actinomycin D, an inhibitor of RNA polymerase transcription, also completely lost KDM3B from the nucleolus. Chromatin immunoprecipitation (ChIP) assays suggests that KDM3B binds to rRNA genes and qPCR data reveal that treatment with LMB causes decreased transcription of rRNA genes. Collectively, these data suggest that interaction with Crm1-RanGTP regulates KDM3B-mediated histone demethylation and rRNA gene transcription. We therefore conclude that interaction of KDM3B with Crm1and RanGTP represents a novel, transport independent mechanism through which the Ran pathway controls histone demethylation-mediated gene expression.

30 A behavioural analysis of fly colour vision

Krishna V. Melnattur, Randall Pursley, Tom Pohida, Chi-Hon Lee

We are interested in understanding how chromatic information is represented in the brain. The visual world is represented in the eye as a pattern of activation of photoreceptors of differing spectral sensitivities. However, the neural connections and computations that transform this pattern of photoreceptor activation into a colour percept are as yet unclear. The sophisticated genetic toolkit and range of specific behaviours make *Drosophila* an attractive model system to explore these questions.

The photoreceptors in the *Drosophila* compound eye are well characterised, and divided into separate classes based on their relative position in the ommatidium and their pattern of opsin gene expression. They consequently respond to a wide range of wavelengths from the UV to the visible range. However, the contributions of specific classes of photoreceptors and their downstream neural circuits to colour vision remain unknown. We plan to use genetic tools developed in the lab to manipulate activity of specific classes of photoreceptors and their partner medulla neurons and thereby dissect the neural circuitry for colour vision in *Drosophila*.

Towards this end, we are developing a novel aversive conditioning based behavioural assay to probe colour vision in *Drosophila*. Single flies are glued to a minutien pin and magnetically tethered in an arena surrounded by an octagonal array of 'blue' (470nm) and 'green' (528nm) LED panels. This arrangement allows the flying fly rotational freedom about a fixed vertical axis. The position of the fly is continuously monitored with custom software, and an infra-red laser is used as punishment to train the flies to avoid either 'green' or 'blue'. We will present our progress towards developing a training regimen, and discuss potential future directions.

31 An N-terminal truncated carboxypeptidase E splice isoform induces metastasis by activating nedd9 and other metastasis inducing genes.

Saravana R.K. Murthy, Terence K. Lee, Niamh X. Cawley and Y. Peng Loh

Cancer mortality is often from metastatic disease rather than the direct effect of the primary tumor. Identification of metastasis inducing genes may offer valuable mechanistic insight for guiding specific therapeutic strategies. We report here that the carboxypeptidase E gene (CPE) is alternatively spliced in human tumors to yield an N-terminal truncated protein (CPE-DELTA-N) that drives metastasis. CPE-DELTA-N mRNA was elevated in human metastatic colon, breast and HCC cell lines. Suppression of CPE-DELTA-N expression in these cell lines by si-RNA significantly inhibited their growth and invasion. To confirm these observations in vivo, an orthotopic nude mouse model was established. The mice implanted with a tumor derived from HCC cells transfected with si- CPE-DELTA-N RNA in the liver did not show tumor growth or metastasis, compared to scrambled controls. In HCC cytosolic CPE-DELTA-N protein was translocated to the nucleus and upregulated the expression of neural precursor cell expressed, developmentally downregulated gene 9 (Nedd9), through interaction with histone deacetylase (HDAC) 1/2. Inhibition of HDAC activity by the HDAC inhibitors suppressed expression of NEDD9, without effecting CPE-DELTA-N expression. The enhanced invasive phenotype of HCC cells stably transfected with CPE-DELTA-N was suppressed when Nedd9 was silenced by si-RNA. cDNA Microarray studies of HCC cells overexpressing CPE-DELTA-N showed elevated expression of 27 genes associated with metastasis such as Nedd9, claudin 2 (cldn2), carcinoembryonic antigen-related cell adhesion molecule 5 (ceacam5), matrix metallopeptidase 1 (mmp1), plasminogen activator (plat) and inositol 1,4,5-trisphosphate 3-kinase A (itpka), while 30 genes associated with tumor suppressor function, which included insulin-like growth factor binding protein 5 and 3 (igfbp5 and igfbp3) and h19 were down-regulated. These data were further confirmed by qRTPCR. Robust increase in the levels of protranscriptional acetylated histone H3 and H4 in these cells suggest epigenetic regulation of downstream genes by CPE-DELTA-N. Thus CPE-DELTA-N induces tumor metastasis by regulating NEDD9 and other metastasis associated genes expression via interaction with HDAC complex. In clinical studies of 14 patients with thyroid papillary carcinoma, resected tumors having very high copy numbers of CPE-DELTA-N mRNA was correlated with metastasis. Thus CPE-DELTA-N may be a potentially useful biomarker for diagnosing metastasis.

Saravana is also a Fellow Speaker.

32 Identification of Adaptor Protein Complex-4 (AP-4) Interactors

Kathryn E. Tifft and Juan S. Bonifacino

Many transmembrane proteins are transported between specific cellular organelles via amino acid signals that bind to adaptor proteins (AP) complexes. AP complexes link the transmembrane cargo proteins to the cellular machinery required to mediate vesicle targeting to specific membranes. Adaptor complexes are composed of two large subunits (beta and alpha, delta, gamma, or epsilon), a medium subunit (mu), and a small subunit (sigma). AP-4, the least well-characterized adaptor complex, localizes to the Golgi and mediates trafficking from Golgi to endosomes. While the AP-4 complex is ubiquitously expressed, knock-out mice with a deletion of the AP-4 beta subunit show neurological defects and human patients with deletion of the epsilon subunit or mutation of the Mu4 subunit have spastic cerebral palsy suggesting that AP-4 function is most important in the brain. In addition, known AP-4 cargo proteins amyloid precursor protein (APP) and AMPA receptors have important roles in neurons: APP is the source of the amyloid beta peptide that accumulates in the brains of patients with Alzheimer's disease and AMPA receptors play a role in synaptic plasticity.

Although several cargo proteins have been identified, the accessory proteins that bind AP-4 and mediate sorting and trafficking events, including the vesicle-forming protein coat, are unknown. Identification of AP-4 accessory proteins is an important step in understanding the mechanisms of AP-4 function. Therefore, we performed yeast-two hybrid screens with regions of the epsilon and beta subunits that revealed several interesting candidate binding partners. Interestingly, the confirmed interactors Rab4 and TMEM59L have both been previously linked to trafficking of APP. In addition, we are using affinity chromatography approaches based on epitope tagging and overexpression of the four subunits of the AP-4 complex to identify novel interactors by mass spectrometry. With confirmed interactors, we are testing the hypothesis that the interaction of AP-4 with novel partners is important for the trafficking of cargo proteins, including APP and AMPA, from the Golgi to endosomes. Further characterization of novel AP-4 protein interactions will reveal the molecular mechanisms of AP-4 mediated transport and help elucidate the role of AP-4 in normal brain function, Alzheimer's disease, and spastic cerebral palsy.

33 Suppressing tumourigenicity of prostate cancer cells by inhibiting osteopontin expression

Yu Zhang

Osteopontin (OPN) expression is increased in prostate cancer cells. This study investigated the possibility of using the increased OPN as a target to suppressthe tumorigenicity of prostate cancer cells. Small interference RNAs against OPN were transfected into highly malignant prostate cancer cells, DU145, which express high levels prior to transfection, to establish OPN-suppressed clones. Compared with the control transfectants generated by scrambled RNA, suppressed expression of OPN significantly inhibited cell invasiveness and anchorage-independent growth. Similar results were obtained from in vivo experiments. OPN-suppressed transfectants significantly reduced the average size of subcutaneous tumors after inoculation into nude mice. When the levels of OPN were analyzed in the tumors produced by OPN-suppressed transfectants, it was found that the suppression of OPN was significantly associated with reduced tumor size. This result suggests that OPN could be an effective target for therapeutic tumor suppression in prostate cancer.

34 Critical role of *Saccharomyces cerevisiae* translation initiation factor eIF4B in stimulating mRNA-ribosome association

Fujun Zhou and Alan Hinnebusch

Almost all eukaryotic cellular proteins are synthesized by a cap-dependent translation initiation pathway, in which a 43S preinitiation complex (**PIC**, consists of 40S ribosomal subunit, initiator tRNA and many initiation factors) attaches to the 5' cap of the mRNA, scans downstream and starts translation at the first AUG codon encountered. Many essential steps of translation initiation, such as mRNA-43S association and ribosomal scanning, are not well understood, due to the lack of information about the molecular functions of the translation initiation factors involved, including eIF4B. eIF4B is a subunit of the cap-binding protein complex eIF4, which also includes a cap recognizing subunit eIF4E, large scaffolding subunit eIF4G, and DEAD box RNA helicase eIF4A. The eIF4B is one of the least characterized initiation factors, and its functions in translation initiation in living cells are unclear.

To characterize the in vivo function of eIF4B in budding yeast, known as Tif3, we have successfully deleted the *TIF3* gene. The *tif3* deletion mutant is not lethal, but confers significant slow growth (Slg$^-$) and cold sensitive (Cs$^-$) phenotypes. By attempting to complement these phenotypes with mutant tif3 variants, we found that the most important domain for supporting Tif3 function in vivo is an array of 7 repeats of 26 amino acids located in the middle of the protein, and a conserved motif in each repeat, D(W/F)XXXR, is critical for supporting cell growth. Surprisingly, the adjacent RNA recognition motif (RRM) in Tif3 is largely dispensable for complementation. This is surprising considering a current model that the RRM of mammalian eIF4B mediates ribosome-mRNA attachment by binding simultaneously to mRNA, through an Arg-rich RNA binding domain, and to 18S rRNA via the RRM. We have explored the biochemical function of Tif3 in vivo using a powerful approach developed by our lab, in which native 43S-mRNA PICs are stabilized by cross-linking cells with formaldehyde, and then resolved by ultracentrifugation through a sucrose gradient. Interestingly, 43S association of several mRNAs was found to be dramatically reduced in the *tif3* mutant cells, indicating a critical role for Tif3 in stimulating mRNA-40S attachment in vivo. Importantly, these in vivo findings are consistent with our in vitro results, obtained using a fully reconstituted system, indicating that Tif3 is indispensable for mRNA-43S association.

35 Electrophysiological study of color vision circuit in *Drosophila* optic lobe

Mingmin Zhou

Drosophila is classical model organism because of its genetic toolbox and behavioral richness. *Drosophila* visual system has served as a model system for studying the processing of visual information, such as motion and color. Using genetic and electrophysiological methods, previous studies have found that on and off neurons in *Drosophila* motion vision. In contrast, little is known about the mechanism of color vision in *Drosophila*. Our previous behavior studies suggest that the medulla, an analogous structure of the vertebrate inner plexiform layer, involves in color vision. Our anatomical studies revealed that a subset of transmedulla neurons, which are analogous to vertebrate ganglion cells, receive inputs from multiple color channels and therefore likely serve as color-opponent cells. In order to explore the mechanism of color vision in *Drosophila*, we have developed novel genetic drivers, which identify specific classes of medulla neurons. I recently establish electrophysiological setup to further study the function of these neurons in color processing in vivo. During last year, I focused on establishing the setup and whole-cell patch technique. Flies are sensitive to blue, green and UV. In future, I will further study the physiological characteristic of medulla neurons to different wavelength of light.

36 Adrenal hyperplasia among adolescent patients with polycystic ovarian syndrome

Evgenia Gourgari, MD, and Constantine Stratakis MD, D(med)Sci.

Polycystic ovarian syndrome (PCOS) is a heterogeneous group of disorders presenting with hyperandrogenism in adolescents and young women. The etiology of this condition remains unknown, despite its many identified links to insulin resistance, hypertension and metabolic syndrome, as well as its potential connection to adrenogenital disorders, such as the various forms of congenital adrenal hyperplasia (CAH). We propose that there is a subgroup of patients with PCOS who actually have non-CAH primary forms of bilateral adrenocortical hyperplasia (BAH). To investigate this possibility, we propose to study the hypothalamic-pituitary-adrenal axis (HPAA) over the next 2 years in 100 young girls and women (ages 16 to 25 years) that we will compare to 30 age- and race-matched normal females. Patients will be recruited primarily (although not exclusively) from a busy New York City clinic run by the Pediatric Endocrine Division at the Infants and Children's Hospital of Brooklyn at Maimonides and SUNY Downstate. All patients will undergo standard testing of the HPAA including oral low- and high-dose dexamethasone (DEX)-suppression testing (Liddle's test). "Paradoxical" rise of cortisol and/or other steroid metabolites in response to DEX is considered a sensitive test for the diagnosis of BAH. Patients with such responses will be molecularly investigated for the known causes of BAH (*GNAS, PRKAR1A, PDE11A*, and *PDE8B* mutations). The goal of this study is to identify any possible contributions of the BAH phenotypes and genotypes to the pathophysiology of PCOS, a yet unknown factor in the etiology of this multifaceted disorder.

37 A novel frame shift mutation in the GHRH receptor gene in familial isolated GH deficiency without anterior pituitary hypoplasia

Rugia Shohreh, Roya Sherafat-Kazemzadeh, **Youn Hee Jee**, Ari Blitz, Roberto Salvatori

Background: Mutations in the genes encoding for GH-releasing hormone receptor (GHRHR) and Growth hormone (GH1) are the most common cause of familial isolated GH deficiency (IGHD). GHRHR mutations are often associated with anterior pituitary hypoplasia (APH). We searched for GHRHR mutations in a consanguineous family with father and three of five siblings with IGHD, but with no obvious MRI evidence in any of the children (one of them had MRI at two different ages: 5 yr and 8 months and subsequently at 14 years and 5 months).

Objective: To find the cause of IGHD in a family without APH.

Methods: We sequenced the whole coding regions and the intron-exon boundaries from peripheral genomic DNA of the index patient. After identifying a novel mutation in the GHRHR, we sequenced the region of interest in the all the other members of the family.

Results: The father and the three affected children were homozygous for a new frame-shift mutation in the coding sequence of exon 4 (corresponding to the extracellular domain of the receptor) (c.391delG) that places the downstream sequence out of frame. The mother and two unaffected siblings were heterozygous for the mutation.

Conclusions: We describe a new mutation in the GHRHR in a family with IGHD. The lack of frank APH even in one of the children who underwent MRI at two different ages stresses that GHRHR mutations must be suspected in familial IGHD even in the absence of this MRI finding.

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Distinct electroencephalographic desynchronization reveals the presence of a mirror mechanism in newborn monkeys

Annika Paukner and Stephen J. Suomi

Newborn infants of humans and other primate species engage with and respond appropriately to social stimulation provided by a caregiver. How does the newborn primate brain encode and process complex social stimuli such as facial gestures? According to the mirror neuron hypothesis, observation and production of actions activates shared cortical motor representations. Using electroencephalography (EEG) in twenty-one newborn rhesus macaques, we measured brain activity recorded from electrodes positioned over anterior scalp locations during both observation and production of facial gestures, and found significant desynchronization in the 5-6 hertz range. These results demonstrate the presence of a mu-rhythm at birth, which reflects the activation of the sensorimotor cortex and is thought to be an early signature of the mirror mechanism. Thus, the basic elements of this mechanism operate from the very first days of perinatal life and are likely to contribute to the capacity to encode and respond to social stimuli.

39

Targeted Pharmacotherapy for the Leptin Resistance of Bardet-Biedl Syndrome

Jack A. Yanovski, **Roya Sherafat-Kazemzadeh**, Joan C. Han, Leslie G. Biesecker, Diane DellaValle, Amber Courville

Background: Bardet-Biedl syndrome (BBS) is a rare (1/100,000) genetically heterogeneous disorder with retinal dystrophy, post-axial polydactyly, developmental delay, renal abnormalities, and obesity. Mouse models of BBS protein hypofunction, which recapitulate features of the human phenotype, showed an altered protein/vesicle trafficking in leptin-sensing hypothalamic neurons. Such mice showed high serum leptin which precedes obesity onset and leptin administration does not induce the expected increase in hypothalamic STAT3 phosphorylation or POMC. BBS1 protein can physically interact with LepR and it was shown that loss of BBS proteins perturbs LepR trafficking. Furthermore, melanocortin receptor agonist administration reduces food intake and body weight in $Bbs^{-/-}$ mice, implying that the melanocortin receptor signaling pathway (downstream of leptin signaling) is intact. Taken all together, these data is indicative of diminished responses in leptin receptor (LepR) at the level of appetite-regulating POMC neurons. Human studies on BBS patients confirmed leptin resistance similar to that observed in $Bbs^{-/-}$ mice, with two-fold higher leptin than expected for their degree of adiposity compared to BMI-matched controls.

Medications that stimulate hypothalamic POMC release, such as Bupropion (BUP) have limited effects on body weight, likely because of compensatory autoinhibitory feedback by endogenous opioid-like β-endorphin. However, recent murine data showed that combined administration of BUP+NAL stimulates hypothalamic POMC neurons in-vitro more than BUP alone and decreases food intake [5]. Human studies of BUP+NAL have also been able to produce clinically-significant weight loss in non-syndromic obese humans (3.7-7.4% of body weight).

Hypothesis- We hypothesize that administration of BUP+NAL in BBS-type ciliopathies will be uniquely efficacious in decreasing food intake and body weight because such treatment will increase POMC neuron activity; hence, producing anorexic effects and bypassing impaired LepR intracellular trafficking.

Subjects & Methods- We will carry out a 24-week pilot trial to study the effect of Bupupropion+Naltrexone combination therapy on adiposity, energy intake, satiation/satiety, and metabolism in 20 obese BBS patients and 20 obese non-syndromic controls (age>18 yrs; BMI>30 kg/m2). The starting dosages for the drugs will be BUP SR 90 mg/d+NAL SR 8 mg/d and if there is no major adverse effect, we will titrate to Bupropion SR 360 mg/d + Naltrexone SR 32 mg/d. Safety, tolerability and pharmacokinetics of the drugs will be studied. The primary efficacy outcome will be change in body weight, with changes in body composition, metabolic profile, and both energy expenditure and intake during test meals as secondary outcomes. Changes in variabes from the intervention will be compared between BBS patients and non-syndromic obese patients.

Should this pilot study have promising results for safety and efficacy of BUP+NAL, data will be used to design a randomized, placebo controlled trial to further study the dosing and effectiveness of this drug combination.

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Creating isometric surfaces from confocal images: Where to draw the line

Jeremy Swan and **Nichole Jonas**

Rapid prototyping is useful for the fabrication of custom parts in conducting experiments. It can also be used to create physical 3D models, which help elucidate and convey information about biological structures and their function. Amira, Imaris and Osirix are a few of the software packages available to the scientific community for viewing imaging data and generating object files, which can be printed with a rapid prototype printer. We will explore how to prepare electron tomography and confocal microscopy data using Amira and Imaris, and how to prepare nuclear imaging data with Osirix, with the ultimate goal of creating rapid prototype models.

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Bias in Diffusion Tensor-Derived Quantities Depend on the Number of DWIs Composing the DT-MRI Dataset.

F. Tannazi, L. Walker, M. Curry, and C. Pierpaoli

Diffusion tensor eigenvalues and anisotropy indices derived from them, such as the fractional anisotropy (FA), are common metrics used in clinical MRI research. Understanding how experimental factors affect the statistical properties of these quantities is important for proper statistical analysis of DTI data. Mathematical simulations have shown that noise in the diffusion weighted images (DWIs) produces biased mean values of FA and of the eigenvalues, while the Trace of the diffusion tensor, Trace(D), is relatively immune from bias [1]. Measurements in isotropic media are more affected than those in anisotropic media [1]. It has been shown that the variance of tensor-derived quantities shows an undesirable dependence on directions if fewer than about 20-30 directions are sampled [2]. Here we explore the effect of the number of images comprising the DTI dataset on the statistical properties of DT-derived quantities. We hypothesize that reducing the number of DWIs composing the dataset will lead to an overall noisier experimental design that will produce consequences on the diffusion tensor derived quantities. To validate this hypothesis, for one of the sites in the NIH Pediatric Neuroimaging Study of Normal Brain Development, we partitioned each DTI dataset that were collected on an isotropic water phantom over 2.4 years in this study into four subsets and computed tensor-derived quantities for each subset and the original complete set. We then used a paired t-test to compare the average of the values obtained by the four subsets with the corresponding values obtained from fitting the complete set. Additionally, we performed Monte Carlo simulations similar to what was proposed in [1] but in our case keeping SNR constant and varying the number of DWIs in the dataset. Paired t-tests of the isotropic phantom data showed significant differences for Trace(D), FA, Eig1, and Eig3 ($p<0.0001$) between the mean values of the four subsets vs. the complete set. Trace(D) and FA are significantly higher for the mean values of subsets compare to the complete set. While there is no significant bias in Eig2 ($p=0.164$), there is a positive bias for Eig1 and negative bias for Eig3. The results of Monte Carlo simulations showing an underestimation of Eig3 and an overestimation of FA and Eig1 as the number of images in DTI estimation is reduced. To the best of our knowledge the observation that bias in tensor-derived quantities can be modulated by varying the number of DWIs from which the diffusion tensor is estimated has not been reported previously. For some tensor-derived quantities, in particular for FA, the level of bias is remarkably high even when using a large number of DWIs. This effect shows striking similarities to what has been described by varying the SNR of DWIs in previous studies [1,2]. A unifying interpretation could be that rather than considering the SNR of the individual images, one should consider the SNR of the entire experiment.

1. Pierpaoli C., et al. *Magn Reson Med.* 36:893-906 (1996)
2. Jones D.K., et al. *Magn Reson Med.* 51:807-815 (2004)

42 In vivo detection of cancer biomarkersusing fluorescence lifetime imaging

Yasaman Ardeshirpour, Rafal Zielinski, Victor Chernomordik, Jacek Capala, Gary Griffiths, Amir Gandjbakhche, Moinuddin Hassan

One of the most important factors in choosing a treatment strategy for cancer is characterization of biomarkers in cancer cells. Particularly, recent advances in Monoclonal AntiBodies (MAB) as primary-specific drugs targeting tumor receptors show that their efficacy depends strongly on characterization of tumor biomarkers. Assessment of their status in individual patients would facilitate selection of an optimal treatment strategy, and the continuous monitoring of those biomarkers and their binding process to the therapy would provide means for early evaluation the efficacy of therapeutic intervention.

In this study we have shown in live animal that the fluorescence lifetime can be used to detect the binding of targeted optical probes to the extracellular receptors on tumor cells in vivo. We attached Near-InfraRed (NIR) fluorescent probes to Human Epidermal Growth Factor 2 (HER2/neu) specific affibody molecules and used our time-resolved optical system to compare the fluorescence lifetime of the optical probes that were bound and unbound to tumor cells in live mice. The results show that the fluorescence lifetime in our model system can be used as specific marker of binding process.

43 Mathematical modeling of clathrin mediated endocytosis

Anand Banerjee, Alexander Berezhkovskii, Ralph Nossal

Clathrin mediated endocytosis (CME) is a process by which eukaryotic cells internalize various macromolecules. It is of fundamental importance to organisms in a number of ways, including nutrition, cholesterol metabolism, hormone responsiveness, and the passive acquisition of immunity during fetal and neonatal development.

CME starts with the assembly of a protein coat on the inner membrane of the cell, which leads to the formation of small invaginations called clathrin coated pits (CCPs). After recruiting cargo, a CCP grows in size and invaginates further, until it is joined to the membrane by a narrow neck. Finally, the neck is pinched off and the cargo-containing clathrin coated vesicle (CCV) is taken into the cell for processing.

Although the molecular players involved in CME are well recognized, the kinetics of CCP assembly is still poorly understood. The reason is that complete understanding of CCP assembly requires coupling of information on protein-protein and protein-lipid interactions with a description of the mechanics of the cell membrane and protein coat deformation. Studies on CME so far have not attempted to account for this coupling.

To address this problem, we model CCP assembly as a nucleation phenomenon. The inputs to our model are the protein-protein and protein-lipid association/dissociation rate constants, and the free energy of formation of CCPs. We obtain the rate constants from biochemical experiments, and calculate the free energy using elasticity theory. Our results show that the formation of a CCV from a CCP can be understood as a phenomenon where a CCP of small size crosses a free energy barrier in order to grow and form a CCV. In the absence of cargo, the free energy barrier is too high and most of the CCPs are “abortive”, i.e., they grow only partially and then disassemble. The model accurately reproduces the statistical properties of abortive pits, such as their lifetime and size distribution. We also find that the free energy barrier is lowered in the presence of cargo, so a CCP can transform into a CCV.

Our model combines detailed molecular level information with the shape and mechanical properties of a CCP to build an integrated theory of CME. It provides insights which are not obtainable through biochemical experiments alone, and therefore is a complimentary and significant step towards fully understanding CME.

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Sprouty proteins and RTK antagonism in the zebrafish lateral line primordium

Damian Dalle Nogare and Ajay Chitnis

The lateral line primodrium migrates caudally along the trunk of the embryo, periodically generating and depositing epithelialized rosettes which will go on to form the mature neuromasts of the lateral line. Within the migrating posterior lateral line primordium (pLLp), fibroblast growth factor (FGF) signals are thought to induce both the specification of a sensory hair cell by driving expression of the proneural gene atohLa as well as the morphogenesis of a cellular rosette surrounding this hair cell precursor. The pLLp must therefore coordinate two complex morphogenetic processes: cell migration and rosette morphogenesis. In the pLLp, FGF signaling also drives the expression of sprouty genes, which typically act as feedback inhibitors of FGF signaling. We show that in the primordium, loss of sprouty function leads to a surprising decrease in expression of the FGF target genes pea3 and. atohTa, suggesting that sproutys are not acting as feedback inhibitors of FGF-dependent gene expression. Instead, they may act to antagonize alternate pathways downstream of RTK signaling which promqte migratory behavior. In this manner, by driving sprauty expression, FGF signaling poth promotes the formation of rosettes and prevents the migratory behavior whicfr predominates at the leading edge and which may interfere with formation of stablp epithelial structures.

45

Evidence that variation in adult body size among mammalian species is achieved by modulating the pace of a growth-limiting genetic program

Angela Delaney, Julian CK Lui, Geoffrey Rezvani, Patricia Forcinito, Vasantha Padmanabhan, and Jeffrey Baron

Body size varies enormously among mammalian species. In small mammals, body growth is typically suppressed rapidly, within weeks, whereas in large mammals, growth is suppressed slowly, over years, allowing for a greater adult size. We recently reported evidence that body growth suppression in rodents is caused in part by a juvenile genetic program that occurs in multiple tissues simultaneously and involves the downregulation of a large set of growth-promoting genes (1, 2). We hypothesized that this genetic program is conserved among mammalian species but its time course is evolutionarily modulated such that it plays out more slowly in large mammals, allowing for more prolonged growth.

To test this hypothesis, we used microarray analysis to compare gene expression in kidney and lung of 1, 4, 8 wk-old mice; 1, 5 wk-old rats; and fetal day 90, 10 wk postnatal, and adult sheep. In all 3 species, we found a large set of genes downregulated with age (false discovery rate < 0.1) in both lung and kidney. The set of downregulated genes was similar in the 3 species; the number of overlapping genes between each pair of species was far greater than expected by chance ($P < 0.001$, Chi square test), indicating evolutionary conservation of the program.

To compare the pace of the program, we studied the 183 genes that were downregulated in both organs of all 3 species. In mouse, the greatest declines occurred by 4 wk of age (45 +/- 2 % expression at 4 wk relative to 1 wk, kidney; 47 +/- 2 %, lung; mean of 183 genes +/- SEM), followed by a slower decline (38 +/- 2 % at 8 wk relative to 1 wk, kidney; 45 +/- 2 %, lung). In rat, higher relative expression was seen at 5 wk (50 +/- 1 % expression relative to 1 wk, kidney; 60 +/- 1 %, lung;) than in mouse at 4 wk, indicating a more gradual decline in rat ($P < 0.001$). In sheep, the declines appeared to occur even more slowly; large declines occurred between 90 d gestation (a greater age post-fertilization than 1-wk-old mouse or rat) and 10 wks of age (51 +/- 2 % expression relative to FD90, kidney; 54 +/- 2 %, lung), and this decline continued after 10 w (46 +/- 2 % expression in adult relative to FD90, kidney; 49 +/- 2 %, lung; $P < 0.001$, kidney and lung) . Additional time points are being analyzed to confirm this pattern. The data suggest that a growth-limiting genetic program is conserved among mammalian species but that its pace is modulated to allow more prolonged growth and therefore greater adult body size in large mammals.

1. Finkielstain GP, et al. *Endocrinology* 150:1791-800, 2009
2. Lui JC, et al. *FASEB J.* 24:3083-92, 2010

46 Real Time Functional Brain Imaging using Principal Component Analysis on Near Infrared Spectroscopy Data

Jana M. Kainerstorfer, Laleh Najafizadeh, Franck Amyot, Jason D. Riley, Paul D. Smith, and Amir H. Gandjbakhche

Near Infrared Spectroscopy (NIRS) is an emerging imaging technology for measuring brain functions non-invasively in humans. By placing illumination fibers on the head and surrounding them with detector fibers, light reflected from the brain can be captured. When using two wavelengths, brain function can be assessed in means of oxygenated (HbO) and deoxygenated (Hb) hemoglobin. In order to calculate these values, a light tissue interaction model is generally used, such as the modified Beer-Lambert law. However, it has been shown previously that the use of this model can lead to erroneous results, dependent on the wavelengths used as well as the geometry of the head imaged. We are proposing an alternative method for analyzing NIRS data, which is based on Principal Component Analysis (PCA), for extracting hemoglobin values model independent and in real time. We have previously explored PCA on multi-spectral skin data, where the underlying principles are the same as in NIRS. Applied to skin data, we have shown that eigenvector 1 corresponds to HbT, eigenvector 2 to HbO, and that the calculated eigenvectors do not deviate between subjects. Given the stability in skin results, our data suggest that there is an underlying mechanism, which allows extraction of HbT and HbO specific eigenvectors, which are wavelength dependent, but most importantly, tissue independent. In order to evaluate this hypothesis, we applied the skin eigenvectors to NIRS data, recorded from the prefrontal cortex. PCA was applied to the NIRS data and results were compared to calculated values using the modified Beer-Lambert law. Model based reconstructed data shows a local increase/decrease in HbO/HbT, coinciding with results reported in the literature. Remarkably, PCA converted data shows the same trend, with eigenvector 1 and 2 corresponding to HbT and HbO respectively. Our data thus supports the hypothesis of using tissue independent eigenvectors for extracting HbT and HbO, making PCA a promising tool for assessing hemoglobin values in NIRS data.

47 Assessment of Functional Brain Activation Using A NIRS/EEG Multimodal System

Laleh Najafizadeh, Jana M. Kainerstorfer, Andrei Medvedev, Franck Amyot, Jason D. Riley, and Amir H. Gandjbakhche

It has been estimated that more than 300,000 U.S. veterans of the wars in Iraq and Afghanistan have experienced a mild traumatic brain injury (TBI). Patients with mild TBI generally experience cognitive deficits, including problems with memory, reduced attention, and the inability to concentrate on a single task. These cognitive impairments, if not diagnosed and treated properly, could significantly impact patient's quality of life. Majority of mild TBI patients, however, show normal structural magnetic resonance imaging (MRI)/computed tomography (CT) scans. Therefore, the need for functional brain imaging techniques that could correlate cognitive impairments with brain functionality still remains.

Near-infrared spectroscopy (NIRS) is an emerging technology which uses light in the range of 700 to 1000 nm to noninvasively measure the local changes in cerebral hemodynamic levels associated with brain activity. Compared to other well-established brain imaging modalities, such as functional MRI, NIR instruments are relatively more patient friendly (smaller, less restraining and less expensive). The technology can be combined with EEG to simultaneously monitor both blood hemodynamic and neuronal responses.

As the most reported neuropsychological damages from TBI patients are in the domains of memory, attention, and executive functioning, a task that engages subjects in using their working memory (N-back) was implemented. A NIRS system, with optical probes placed on the frontopolar and left and right lateral frontal areas, was combined with a 128-channel EEG system, for experiments.

Changes in hemoglobin concentrations in conjunction with EEG data identify the areas involved in performing the N-back task. Results show strong activations in right frontopolar region. This study demonstrates the capabilities of optical imaging technique in providing information not only on spatial localization but also on interregional interactions during cognitive processes.

48 A polar probabilistic atlas for MRI-free functional imaging in MNI/Talairach space

Jason D. Riley, Vippin Reddy, Franck Amyot, Laleh Najafizadeh, Jana M. Kainerstorfer, Yasaman Ardeshipour, Victor Chernomordik, and Amir H. Gandjbakhche

Structural and functional brain atlasing play an integral role in neuro-imaging for MRI, CT, PET and other modalities. In modalities such as MRI there are well established brain atlasing approaches such as the Talairach atlas or Montreal Neurological Institute Atlas. In optical imaging there have been various attempts to link these atlases to optical data using Cartesian spaces and complex image registration methods. Here we present a new approach which addresses the central issue in optical atlasing, that optical imaging is not sensitive in a Cartesian space sense, but rather in a radial sense. By so doing we provide a tailored approach which obviates many of the optical atlasing issues, such as depth uncertainty and registration of patient specific models to standard atlases. We also propose to move to a novel probabilistic model for functional imaging more in-line with the acquired data.

49 Role of microtubules in durotaxis

Raimon Sunyer

It is well documented that directed motion of cells is influenced by substrate stiffness. When cells are cultured on a substrate of graded stiffness, they tend to move from softer to stiffer regions—a process known as durotaxis [1]. However, much of this phenomenon remains ill-defined. In particular, it is unclear how cells sense the stiffness gradient and drive polarization. Durotaxis has frequently been described as a focal adhesion/actin phenomenon. Stiffness asymmetry in focal adhesions creates a bias that guides cell movement from softer towards stiffer substrate regions [1]. However, in other kinds of cell migration, microtubules and cell polarization agents are essential. Empirical observation shows that ~90% of the cells on a stiffness gradient are polarized, independently of stiffness gradient strength [4]. This observation suggests that microtubules are potentially important parts of durotaxis. The aim of this project is to study the contribution of microtubules to cells' durotactic response. In the first part of the study, polyacrylamide gels with a gradient of stiffness up to 30 kPa/mm will be produced and characterized. In the second part of the study, we will observe how durotaxis and cell polarization is altered by challenging cells with microtubule targeting agents such as taxol and colchicine.

1. Lo, C.M., et al., Cell movement is guided by the rigidity of the substrate. *Biophysical Journal*, 2000. 79(1): p. 144-152.
2. Gundersen, G.G. and J.C. Bulinski, Selective Stabilization of Microtubules Oriented toward the Direction of Cell-Migration. *Proceedings of the National Academy of Sciences of the United States of America*, 1988. 85(16): p. 5946-5950.
3. Etienne-Manneville, S. and A. Hall, Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKC zeta. *Cell*, 2001. 106(4): p. 489-498.
4. Isenberg, B.C., et al., Vascular Smooth Muscle Cell Durotaxis Depends on Substrate Stiffness Gradient Strength. *Biophysical Journal*, 2009. 97(5): p. 1313-1322.

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Hindered diffusion in polymeric solutions studied by Fluorescence Correlation Spectroscopy

Silviya Zustiak, Ralph Nossal, Dan Sackett

The typical cell is crowded with both charged and neutral molecules which hinder solute diffusion and ultimately impact all cellular processes. For example, crowding has been shown to trigger aggregation of the amyloidogeneic proteins associated with neurotoxicity in Alzheimer's disease. Despite the vast interest in the subject, hindered diffusion in the cell is still poorly understood. In particular, the combined effect of crowding and non-specific binding has rarely been addressed, partly due to the inability to decouple their individual contributions in the heterogeneous cell environment.

We developed a homogeneous in-vitro cell model, with tunable binding and crowding, in order to elucidate their relative roles in solute diffusion. We used Fluorescence Correlation Spectroscopy (FCS) to measure the diffusion of a charged protein, Ribonuclease A (RNase), in dextran solutions of various charges (acting as binders) and concentrations (acting as crowders). Originally designed for measurements in dilute media, FCS has recently been employed in studies of diffusion in crowded media. However, such diffusion is mainly interpreted as anomalous, with little insight into the specific causes for the anomaly. We used a more complex two-component FCS analysis, which allowed us to extract quantitative binding data. The non-specific nature of the binding was confirmed by incremental addition of salt, which prevented binding by shielding the RNase and dextran charges. Moreover, we validated our FCS results by a separate, well established technique - ultrafiltration.

In agreement with existing data, we observed an overall 5 fold decrease in RNase diffusivity at the highest concentration of dextran, where binding accounted for 75% and crowding for 25% of the decrease. Interestingly, binding decreased RNase diffusivity by 32% even at 0.4 μM dextran. In contrast, crowding affected diffusivity only above a crowder concentration of 20 μM. Further analysis revealed that 100 μM crowder, as compared to 1 μM binder, was needed to achieve equivalent reduction in RNase diffusivity. However, the data suggested that at a higher crowder concentration (300 μM,similar to that in the cell), crowding would overpower the effect of binding.

This is the first study to highlight the relative contribution of non-specific binding and crowding to hindered diffusion in crowded and charged media and thus can facilitate future understanding of molecular transport implicated in key cellular processes.

Silviya is also a fellow speaker.

51 Genetic Screening for Enhancer Loci of Intracranial Hemorrhage in Zebrafish.

M. G. Butler, A.V. Gore, D. Castronova, A. E. Davis & B.M. Weinstein

Stroke is the third leading cause of mortality in the U.S.A. Approximately 10% of stroke cases result from intracranial hemorrhage (ICH), in which weakened blood vessels in the brain rupture into the surrounding tissue. Although less common than occlusive stroke, ICH is associated with disproportionately high rates of death and long-term disability. In humans, mutations in any one of the three *Cerebral Cavernous Malformation* (CCM) genes result in dominant ICH. The proteins encoded by these CCM loci physically interact, suggesting each functions in a common pathway that when perturbed causes ICH. However, the penetrance, onset, and expressivity of CCM*s* are highly variable even within affected families, suggesting that additional genetic factors may be modifying individual susceptibility to CCM lesions.

Most of the genetic factors associated with ICH in humans, like *ccm1*, are conserved in zebrafish and when disrupted result in ICH. We are utilizing the advantages of the zebrafish for forward genetics to discover additional *ccm* pathway genes. Our screen uses phenotypically normal *ccm* mutant heterozygotes which are crossed to mutagenized fish to identify dominant enhancers of the ICH phenotype. Human orthologs of candidate genes identified in this first-ever forward genetic screen for stroke susceptibility loci will be examined to determine whether they also act as modifiers of stroke susceptibility in human ICH patients, potentially leading to improved diagnosis and treatment.

52 Constitutive lymphoid expression of the nuclear form of RNase H1 is associated with a developmental bottleneck at the pro-B cell stage of B cell differentiation

Lina A. Gugliotti, Kiran B. Sakhuja, Hongsheng Wang, Susana M. Cerritelli, Herbert C. Morse III, and Robert J. Crouch

The development of B lymphocytes and the process of lineage determination are initiated by expression of a set of transcriptional regulators leading to V(D)J recombination events initiated by double-strand DNA breaks. Subsequently, these recombinations form DNAs that permit transcription of immunoglobulin genes and translation of the corresponding mRNAs, first by joining the V(D)J DNA sequences, then by recombination, that generates various isotypes of immunoglobulins by class-switch recombination (CSR).

Formation of R-loops, regions containing RNA/DNA hybrid and a displaced single-stranded DNA, have been shown to lead to recombination in bacteria, yeast, HeLa and chick cells. Expression in each of these cases of excess ribonuclease H1 (RNase H1), a class of enzymes that degrade RNA in RNA/DNA hybrids, has ameliorated the deleterious effects and decreased recombinational events associated with R-loop formation. R-loops have been observed following transcription of the switch regions that occurs during CSR. The possibility that R-loops are important in V(D)J recombination has not been addressed, and whether ribonucleases H (RNases H) play a role in this process is still uncertain.

Transgenic (TG) mice that overexpress RNase H1 in B and T cells (M27F7) were employed in this study. FACS analysis of hematopoietic cells from TG mice revealed a decrease in pre-B cells in the bone marrow. The data indicate a block at the pro-B to pre-B stage of B cell development, which may be the result of apoptosis due to the failure to generate a productive VH-D-JH rearrangement and expression of the pre-B cell receptor. A few B cells that successfully passed these checkpoints predominately differentiated into marginal zone and B1a cells in the peripheral lymphoid organs of the TG mice. These data suggest that R-loops are important in H chain gene rearrangement.

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53 Role of Interferon Regulatory Factor 8 (IRF8) in Autophagy

Monica Gupta, Lakshmi Ramakrishna, Dong-Mi Shin, Hebert Morse C and Keiko Ozato

Autophagy has a pivotal role in innate and adaptive immunity. It is involved in the removal of intracellular bacteria and viruses in dendritic cells (DCs) and macrophages. Autophagy also facilitates efficient MHC II presentation to T helper cells by these cells. Factors that regulate autophagy in macrophages and DCs cells are, however, not well understood. Here we found that interferon regulatory factor 8 (IRF8), a transcription factor essential for myeloid cell function, plays a critical role in full execution of autophagy in macrophages and DCs. Pathogen components represented by toll like receptor (TLR) ligands and interferons (IFN) enhance the expression of a series of autophagy-related genes in these cells. We found, by ChIP-on-chip analysis that IRF8 binds to the promoters of multiple autophagy-related genes in DCs. Subsequent analysis found that the expression of many of autophagy-related genes was markedly reduced in IRF8$^{-/-}$ macrophages and DCs. Microscopic analysis revealed that typical LC3 punctate structures induced by IFNgwere much reduced in IRF8$^{-/-}$ cells. Experiments are underway to test the conversion of LC3 I to LC3 II in the presence of IRF 8 and to study the mechanism by which IRF 8 regulates autophagy in these cells.

54 HIV-1 and reactivated coinfecting herpes viruses reprogram the cytokine network in semen of infected individuals

Andrea Introini, Andrea Lisco, Arshi Munawwar, Christophe Vanpouille, Jean-Charles Grivel, Sarman Singh, and Leonid Margolis

Background: In semen, HIV-1 and co-infecting viruses are immersed in a complex network of cytokines that affect and are affected by viral replication. Understanding the complex mechanisms of cytokine-mediated interactions between HIV-1 and co-infecting viruses is required for designing strategies to prevent HIV sexual transmission. Here, we report on the cytokine network in semen and blood of individuals co-infected with HIV-1 and herpesviruses.

Methods: The loads of HIV-1 and of 6 herpesviruses were evaluated in semen and blood plasma of 74 HIV-1^{+} and 33 HIV-1` individuals by real-time PCR. A multiplex bead-based assay was used to measure the levels of 21 cytokines. The cytokine network was defined by calculating Spearman`s rank correlations for all pairwise combinations of cytokines.

Results: EBV DNA was found in semen of 56% HIV-1^{+} (median load (ML) 4 log10 copies/mL) and 0% HIV-1` individuals ($p<0.01$). CMV DNA was found in semen of 70% HIV-1$^{+}$ (ML 6 log10 copies/mL) and 3% HIV-1` individuals (ML 3 log10 copies/mL, $p<0.01$). Concomitant seminal shedding of CMV and EBV was associated with higher HIV-1 load in seminal plasma. In HIV-1` subjects, 9 cytokines were enriched in seminal plasma (IL-1a, IL-7, IL-8, MIP-3a, MCP-1, MIG, IP-10, SDF-1b, TGF-b), 4 in blood plasma (IL-2, IL-16, IFN-g, Eotaxin), while 7 had a similar seminal-blood plasma levels (IL-1b, IL-6, IL-15, MIP-1a, MIP-1b, GM-CSF, TNF-a). In semen of HIV-1^{+} patients 16 cytokineswere upregulated compared to HIV-1` individulas: IL-1a, IL-1b, IL-6, IL-7, IL-8, IL-16, MIP-1a, MIP-1b, MIP-3a, RANTES, MCP-1, Eotaxin, MIG, IP-10, SDF-1b and TGF-b ($p<0.05$). RANTES, Eotaxin, MIG and IP-10 seminal levels were higher in individuals co-infected with HIV and CMV compared to HIV-1^{+} individuals not shedding CMV ($p<0.05$). Analysis of the cytokine network revealed 72 statistically significant correlations in semen of HIV-1^{+} patients vs. 21 significant correlations in semen of HIV-1` individuals ($p<0.01$).

Conclusions: Semen of HIV-1 infected individuals is enriched in cytokines which may affect the viral replication. HIV-1 infection is associated with a local reactivation of CMV and EBV in the male genital tract that may trigger further changes in seminal cytokines spectra and directly influence HIV-1 transmission. The intricate relationships between HIV-1 replication, production of specific cytokines and CMV/EBV genital reactivation may determine HIV-1 evolution in semen and its infectivity in the female genital mucosa.

55 Changes in microRNA expression during juvenile growth

F. Kamran, A. C. Andrade, G. Rezvani, O. Nilsson, J. Baron and J. C. Lui

Body growth is rapid in young children, but with increasing age, growth gradually slows and eventually ceases in multiple organs because of a progressive decline in cell proliferation, which begins in early life. We previously showed evidence that this decline in proliferation is caused in part by coordinated postnatal downregulation of a large set of growth-promoting genes (Endocrinology 150:1791-800, 2009; FASEB J 24:3083-92, 2010). We hypothesized that microRNAs help orchestrate this juvenile genetic program. MicroRNAs are endogenous short non-coding RNAs that regulate gene expression by binding to multiple messenger RNAs, consequently blocking translation or stimulating mRNA degradation. MicroRNAs play an important role in regulating a variety of cellular processes, including proliferation. To explore the possibility that changes in microRNA levels help coordinate this genetic program, we compared microRNA expression in juvenile mice at different ages, as growth slows. MicroRNA microarray (Agilent Mouse Genome VI microRNA arrays, targeting 567 microRNAs) was performed in mouse kidney and lung tissue at 1 and 6 weeks of age (n=6 per group) and analyzed using GeneSpring software. A significant change with age was defined by t-test with Benjamini-Hochberg false discovery rate < 0.05 and fold change > 1.3. Because the juvenile genetic program occurs in multiple organs, we focused on the 8 microRNAs that showed concordant upregulation (microRNAs133a, 328 and 7a) or downregulation (microRNAs 106b, 351, 450b-3p, 409-3p and 376a) with age in both organs. Real-time PCR of the mature microRNAs (normalized to Sno234 expression) was used to verify our microarray results. To date we have examined 3 microRNAs in lung at ages 1, 4 and 8 weeks. The results confirmed significant upregulation of microRNA-133a ($P = 0.003$) and microRNA-7a ($P = 0.001$) with age. MicroRNA-106b expression declined but did not reach significance ($P = 0.054$). In conclusion, we have identified specific microRNAs that show changes in expression during juvenile life in both mouse kidney and lung. Whether these microRNAs help coordinate the juvenile regulation of multiple growth-regulating genes and thus drive growth deceleration remains to be determined.

56 The composition of Polycomb group protein complexes at Polycomb response elements during the on/off transcriptional states of engrailed

Kristofor Langlais and Judith Kassis

Early in *Drosophila* development, chromatin is modified in order to stably promote or repress the activity of key genes, in a heritable manner, to maintain established expression patterns into later developmental stages. Polycomb group (PcG) proteins, which interact with sequences called Polycomb Response Elements (PREs), play a major role in the formation of repressive chromatin states. Expression of the segment polarity gene engrailed (en) is regulated by many individually functional PREs, each of which contains binding sites for several different PcG proteins. It remains unclear if PcG proteins are bound to PREs in only the transcriptionally off chromatin state, or if some PcG proteins are bound even in the on state. Chromatin immunoprecipitation (ChIP) studies using larval imaginal discs or tissue culture material have yielded conflicting results on this matter, possibly due to the fact that these samples contain cells in various states of transcription. To clarify these findings and to specifically examine the situation at the en locus, we generated transgenic fly lines that express FLAG-tagged PcG proteins under control of the UAS/GAL4 system. Using various GAL4 driver fly lines, we expressed tagged-PcG proteins specifically in en “on” cells, or in en “off” cells in embryos and imaginal discs. ChIP results demonstrate that all PcG proteins tested are present at the primary en PRE in both the on and off states. However, the results also suggest that one of the PcG proteins, Scm, is present at the PRE in the off state at significantly higher levels, and conversely is less present in the on state, than other PcG members. This observation, suggests that Scm may be a key player in triggering the repressive activity of ubiquitously bound PcG protein complexes.

57 The developmental role of LAT1 as a thyroid hormone and amino acid transporter using a knockout mouse model.

Mitchell, FE; Taylor, PM; Shi, Y-B.

Thyroid hormones (TH) are required for normal development in vertebrates and must be transported into cells in order to access nuclear receptors and effect their genomic actions. Branched-chain amino acids (BCAA) are essential nutrients that are required for protein synthesis and have roles in cell signaling. The LAT1 gene encodes the light-chain transport subunit of a heterodimeric glycoprotein-associated transporter complex that accepts both BCAA and TH as substrates. We have generated a LAT1-knockout mouse line using the Lox-P method. Expression of the LAT1 protein can be prevented globally or in a tissue-specific manner. This has allowed us to explore the functional role of the LAT1 protein during mammalian development.

Wild-type LAT1 is widely expressed and is thought to play a critical role in maintaining BCAA and TH balance in many tissues. LAT1 has been shown to transport TH in a number of tissues and could therefore have a significant role in TH-mediated development. $LAT1^{+/-}$ animals are viable and fertile but the lack of $LAT1^{-/-}$ live births suggests that LAT1 expression is required for embryonic development with $LAT1^{-/-}$ having an embryonic lethal phenotype.

$LAT1^{+/-}$ mice are around 10% smaller than their littermates, with no measurable change in food-intake. Quantitative RT-PCR data confirms that the expression of LAT1 in a number of tissues, including the brain and heart, is significantly reduced. Uptake of the BCAA phenylalanine into diaphragm is significantly reduced by over half in $LAT1^{+/-}$ mice and BCAA concentrations in both liver and skeletal muscle are significantly reduced from control, as determined by High Performance Liquid Chromatography (HPLC). These results would suggest that LAT1 mRNA expression is reduced in $LAT1^{+/-}$ mice, lowering the availability of functional LAT1 protein, and thus reducing the transport activity normally attributable to LAT1. Our current research is focused on determining the role of LAT1 in TH and BCAA-controlled development at both a molecular (eg. regulation of TH-target genes) and tissue (eg. changes in intestinal morphology) level.

58 Vascular-specific translational profiling in Zebrafish

Kathryn Monzo and Brant Weinstein

The development of the vascular system involves the regulation of many processes including the acquisition of endothelial cell fate, the patterning of vessel tracts, and the formation of a closed circulatory system. Many genes required for normal vascular development have been identified, and it is clear that the various aspects of vascular development depend on the integration of many different signaling pathways. Precisely how these pathways interact and the identity of their effector genes are not completely known. Understanding the entire gene expression landscape in whole animals is essential for working out molecular pathways and their effects on cell behavior during normal development as well as during disease processes. Analyzing gene expression from cells in complex tissues such as the vasculature is challenging, and techniques including fluorescence-activated cell sorting (FACS) and laser capture microdissection (LCM) have been employed to address this challenge. Unfortunately, due to the inherent disruptive nature of these techniques, changes in gene and protein expression may be introduced during the analysis that do not represent normal expression. To circumvent these challenges in our studies of the vascular system, we are developing a recently described technique termed translating ribosome affinity purification (TRAP), which identifies cell-specific, polysome-associated, translating mRNAs to be used in zebrafish. Using a vascular specific promoter, we are able to specifically express an affinity tagged ribosomal protein (RiboTag) in endothelial cells of zebrafish embryos. Importantly, we have validated the function of the RiboTag using polysome sucrose gradient analysis and found that the RiboTag co-sediments with other ribosomal proteins in polysomes. In addition, the RiboTag and associated polysomes can be readily immunoprecipitated, and co-immunoprecipitating mRNAs can be purified and analyzed by next-generation sequencing. Our initial goal is to generate TRAP profiles from embryos with a loss of- or gain of function of different signaling pathway components (e.g., VEGF, Notch, Rho GTPase). This analysis will potentially reveal new signaling effectors as well as how these signals are integrated during vascular development. Ultimately, we also also plan to compare TRAP profiles of various mutants with vascular development defects to the known pathways to better understand the molecular basis of the defects. We believe this profiling system has the potential to greatly increase our understanding of normal vascular development as well as the etiology of vascular diseases.

59 Formation and Interpretation of the BMP Morphogen Gradient in the Early *Drosophila* Embryo

Peluso, C., Umulis, D., Kim, Y., O'Connor., M, Serpe, M.

Bone Morphogenetic Proteins (BMPs) regulate dorsal/ventral patterning across the animal kingdom. In the early *Drosophila* embryo, formation of the gradient requires the redistribution of the BMP ligand. This occurs through a conserved shuttling mechanism that involves the activity of the BMP binding protein Short gastrulation (Sog), and the protease Tolloid (Tld). Chordin, the vertebrate counterpart of Sog, cannot promote long-range shuttling of BMP when introduced in flies. Molecularly, these two proteins differ in that Sog processing by Tld is dependent on BMP-binding, while Chordin processing is not. We propose that the BMP-dependence for Tld mediated cleavage, constitutes the basis of Sog's ability to mediate the long-range transport of BMPs.

To test this hypothesis, we characterized residues at the Tld cleavage site of Sog, and mutated them to generate a variant (Sog-i) that can be cleaved independent of binding to BMP. We replaced endogenous Sog with Sog-i and indeed found that the BMP gradient profile was altered in *sog-i* embryos.The peak BMP-signaling domain was expanded, and its intensity was reduced. This impacted cell-fate specification as evidenced by a larger amnioserosa field. Our data suggest that Sog-i was less efficient at transporting BMPs to the dorsal-midline. Also, *sog-i* embryos exhibited increased variability, which may compromise robust developmental patterning of the embryo.

60 Characterization of Polycomb group proteins regulating the silencing of Drosophila *engrailed* gene

Payal Ray and Judy Kassis

Early in *Drosophila* development, the expression of certain genes is silenced in a tissue specific manner. This silencing is mediated by a group of factors known as the Polycomb group proteins (PcG). These proteins bind to specific DNA sequences known as PcG response elements (PRE) in the regulatory region of a given gene. PREs can range from a several hundred to a few thousand base pairs and can be subdivided into smaller fragments with similar activities. Several protein-binding sites are essential for PRE activity. Although several labs have studied PREs from various genes it is still not possible to accurately predict the constituents of a PRE.

The Drosophila *engrailed* gene contains a PRE that has been studied extensively by our group. Within the en PRE a minimal 139 bp region has been identified as essential for the silencing of this gene. My project aims to identify the various proteins that bind to this region and characterize them. I will be using a biotin-streptavidin based pulldown followed by mass spectrometric analysis to identify the various proteins that bind to a small region within this 139bp region.

61 Endothelial-specific Inducible Gene Expression System in Transgenic Zebrafish

Matthew R Swift and Brant M Weinstein

Spatially restricted, inducible gene expression in zebrafish remains elusive despite many available systems for use in mammals and in cell culture assays. Esengil et al. have developed a small-molecule inducible gene expression system derived from the insect-specific ecdysone receptor (EcR) to work in the established Gal4/Vp16-UAS system. No vertebrate EcR ortholog exists and small-molecule activators of EcR have no known activity in vertebrates. A modified form of the *Bombyx mori* EcR is coupled to the DNA-binding domain of the yeast transcription factor Gal4 and a truncated form of the activation domain of the herpes simplex virus regulatory protein VP16. Upon transfection of this chimera (GV-EcRF'), exogenous addition of the steroidal compound Tebufenozide is able to induce 1000-fold induction of UAS-reporters in test systems. Upon placement of the GV-EcRF' system under the control of the vascular-specific promoter *fli1a*, I have been able to demonstrate effective endothelial-specific inducible transgene expression in injected Tg(UAS:Kaede) animals. Additionally, I have established a germ-line transgenic activator line (Tg(Fli1a:GV-EcRF') which allows for EC-specific control of transgene expression at multiple stages of development. Using an established UAS-N1ICD reporter line, I have assayed for defects in hematopoietic and vascular development upon EC-specific activation of Notch signaling at different time-points in development. This system is a powerful tool for zebrafish models of developmental defects by overcoming limitations that occur when transgene overexpression at earlier stages of embryogenesis, or whole embryo transgene overexpression may obscure its role in specific organ or tissue formation.

62 Structural Components and Morphogenetic Mechanics of the Zebrafish Yolk Extension, a Developmental Module

Valerie C. Virta

In order for a vertebrate embryo to attain a functional morphology, it must fundamentally change its shape from a sphere to an elongated rod. While cell movements on the dorsal side of the embryo have been extensively studied, morphogenetic cell behaviors shaping the ventrum have not been described at the same level of detail.

The zebrafish yolk extension ontogenesis module serves as an excellent model for morphogenetic movements reshaping the ventrum because (1) the zebrafish embryo is optically transparent; (2) yolk extension ontogenesis occurs relatively quickly; and (3) the yolk cell isolates the tissues elongating the ventrum from the rest of the embryo. Furthermore, the zebrafish is an ideal taxon in which to perform studies of ventral cell movements, as these results can then be compared both to other teleosts, and other vertebrates. From the core to the cortex of the embryo, three histological compartments comprise the yolk extension developmental module (1) the yolk cell; (2) the mesendodermal mantle; and (3) the embryonic integument.

These structural compartments are hypothesized to interact with one another in a hierarchical manner, resulting in the morphogenesis of the elongated embryonic zebrafish ventrum. Time-lapse videomicroscopy and experimental manipulation show that the yolk mass is a viscoelastic, high-internal-phase emulsion, which resists compression. Moreover, ventral posterior mesoderm separates Kupffer's Vesicle from the yolk cell. Finally, the embryonic integument is contractile and contributes to yolk extension formation. These experiments constitute an initial assessment of the morphogenetic mechanics underlying the zebrafish yolk extension ontogenesis module.

63 Prox1 Influences Neuromast Deposition Frequency and Proliferation in the migrating Posterior Lateral Line Primordium

Kyeong-Won Yoo, Ajay Chitnis

Most cancer deaths are due to the development of metastases, hence the most important improvements in morbidity and mortality will result from prevention (or elimination) of such dissemination or disseminated cancer cells. But the studies for this issue in vivo are not so accessible to investigate the molecular mechanism by which how the events of invasion occur. Many of signaling mechanism of cancer growth and metastasis hijack the mechanism of animal development. Collective cell migration and deposition during zebrafish lateral line development is a good model system to study the molecular mechanism by which it is better understood how the cancer cells are disseminated.

The posterior lateral line primordium (pLLp) periodically generates neuromasts and deposits them from its trailing end as it migrates from the otic vesicle to the tip of the tail. In this study we have examined the role of the homeodomain transcription factor Prox1 in the migrating pLLp. Prox1 is broadly expressed in the pLLp, which contains 2-3 proneuromasts at progressive stages of maturation. Its expression, however, is absent in differentiating hair cells formed by the division of the sensory hair cell progenitor and in the inter-neuromast cells deposited by the migrating pLLp as well.

Knockdown of *prox1* with morpholinos reduced the frequency of neuromast deposition in the pLLp. These changes were associated with reduced BrdU incorporation and expanded expression of Wnt effector *lef1* in the pLLp. In contrast, ETS-transcription factor, *pea3* known to be a target of FGF signaling was significantly reduced. Our results suggest Prox1 facilitates neuromast deposition by both promoting proliferation and the transition from Wnt to FGF signaling in the migrating pLLp.

A

Modulation of DNA condensation by cation valence

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DNA is a highly negatively charged polymer. It is semirigid and self-repelling, occupying an expanded free volume. Poly-cations, however, can condense DNA to less than 1000 times its free volume; allowing several micrometers of DNA be packed into viruses and bacteria of nanometer size. There has been a lot of interest recently in using poly-cations to package DNA into stable nanoparticles for purposes of gene therapy, as well as for generating self-assembling DNA nanostructures. We show that polycation valence is an essential tool for rationally modulating DNA condensation. Though poly-cations of varying valence yield a rich variety of DNA nanostructures, it is not clear what the precise differences are and where in the condensation pathway these differences arise.

We compared the condensation structures produced by trivalent cation spermidine against polyvalent cation polyethylenimine (PEI) (~100^{+}). To check the dependence on DNA size and association kinetics, we used two different DNA: a short linear DNA (1kBp) and a longer plasmid DNA (12.5kBp). Condensates were prepared by incubating DNA and polycation in 50um NaCl for 12 hours (DNA concentration: 1ng/ul; cation:bp ~4). The solution was air-dried onto mica surface for imaging with Atomic Force microscopy.

PEI condensed DNA into nanoparticles of radius ~100nm, whereas spermidine condensed DNA into flowery networks. Shorter DNA gave less compact structures: the networks were more open and the nanoparticle showed an interwoven DNA arrangement. The PEI nanoparticles were smooth.

The PEI condensates remain unchanged on imaging surfaces of opposite charge and hydrophobicity. The spermidine condensates however changed. This suggested that PEI nanoparticles were formed in solution, but spermidine condensates were forming on the surface. Image analysis of the spermidine condensates showed that, inspite of their differing structures, they comprised of fibrils with ~2-3X DNA diameter. To investigate this further, DNA was incubated 10X less spermidine. The imaging surface was now covered with fibrils and simple branched complexes. The fibrils were ~2-3X DNA diameter and independent of the imaging surface, indicating that they are solution features which are probably assembling into flowery networks on the surface during drying at the higher spermidine concentration.

A pathway for DNA condensation was constructed from time-sequential images. The process appears to start with DNA condensing along its length into thicker regions or 'fibrils'. The fibrils are sticky- they undergo head-on, intertwining or parallel association to form simple branched networks. With PEI, the networks densify progressively to form nanoparticles. With spermidine, however, condensation stops at fibril association stage. Therefore when the fibrillar solution is then air-dried for imaging, the fibrils assemble into complex flowery networks on the surface, in a very surface and concentration sensitive manner.

Our observations are in agreement with molecular dynamics and X-ray diffraction studies showing that long polycations have specific, long-lived association with DNA, whereas short polycations have diffuse short-lived associations. Our study shows that cation valence can be used to rationally modulate DNA condensation, and that cations of different valence follow the same condensation pathway but to different extents.

B

Analysis of DNA re-replication dynamics: what can we learn about origins of replication in mammals?

Christelle de Renty and Melvin DePamphilis

In eukaryotes, chromosome duplication is initiated from numerous replication origins that are activated according to specific spatio-temporal programs which ensure the duplication of the genome once and only once in the imparted time before mitosis. In mammalian cells, multiple mechanisms ensure that once activated, an origin will not initiate a new round of replication in the same cycle. As cells exit metaphase and enter into the G1-phase of the cell cycle, the origins of replication are functionalized by the loading and assembly of pre-Replication complexes (preRC: ORC, Cdt1, Cdc6 and MCM proteins), "origin licensing". This process is allowed by a low Cyclin-dependent Kinase (CDK) activity. At the onset of S-phase, CDK activity increases and activates the previously licensed origins leading to the initiation of DNA replication. In the mean time, CDK activity prevents the loading of new preRC, by phosphorylation, ubiquitination and degradation of some of the preRC proteins. Metazoa also express Geminin, a specific inhibitor that binds to Cdt1 and prevents its loading on the chromatin until the next G1 phase. Any failure in these mechanisms can induce DNA re-replication. For example, depletion of Geminin by siRNA induces DNA over-replication in cancer cells, which then exhibit more than 4n DNA content. This re-replication depends on the preRC proteins suggesting that preRC are reloaded and origins re-activated in the same cycle. By using a single molecule approach we are analyzing the dynamics of DNA re-replication. Modified nucleotides (Bromo-, Chloro- or Iododeoxyuridine) are incorporated into DNA during replication or re-replication, followed by their immuno-detection and mapping on single DNA molecules aligned and stretched on microscope glass slides by a process called DNA Molecular Combing. This state of the art technique allows the monitoring of different parameters defining the spatio-temporal program as the speed of replication forks, the density of origins, their distribution, or their mapping when combined with Fluorescent In Situ Hybridization (FISH). The preliminary results suggest that not all the origins of replication are re-activated when cells are induced to re-replicate their DNA and that they show an original distribution. What defines an origin of replication in mammals is still not clear and analyzing the DNA replication versus re-replication dynamics will give us indications on what specifies the loading or reloading of preRC.

C

Generation of induced pluripotent stem cells is regulated by mitochondria within the somatic cell of origin

Kevin Francis, Heinrich Westphal

Induced pluripotent stem cells (iPSCs) have immense potential for disease modeling and regenerative medicine. Questions still surround iPSCs, including the inefficiency of iPSC formation and precise mechanisms regulating cell reversion to the pluripotent state. Recent work has suggested a link between mitochondria, differentiation and embryonic stem cell (ESC) self-renewal. In contrast to somatic cells, mitochondria within ESCs exhibit reduced oxidative capacity, high lactic acid production and less mitochondrial DNA (mtDNA) per cell. However, to what extent mitochondria are affected during iPSC generation and whether they play a role in reprogramming is unclear.

To examine this, mouse fibroblasts were reprogrammed to iPSCs using retroviral transduction of the transcription factors Sox2, Oct-4, cMyc and Klf4. Clones were isolated and characterized for full versus partial reprogramming based on morphology, pluripotent gene expression, viral transgene silencing and germ layer differentiation. Mitochondrial number, activity and function were then examined in fully reprogrammed clones, partially reprogrammed clones, control ESCs and fibroblasts used for iPSC generation. FACS analysis of mitochondrial activity and number, as well as RT-PCR analysis of mtDNA content, demonstrated full reprogramming reduced mitochondria number and content to ESC levels. However, both assays showed insignificant changes when comparing partial clones to fibroblasts. Analysis of oxygen consumption and lactic acid production demonstrated functional mitochondrial reversion as well with full reprogramming, but not partial.

Based on these results, we hypothesized the mitochondrial content and activity within the fibroblast of origin could influence iPSC generation. To test this, fibroblasts were isolated from Oct-4/GFP transgenic mice, sorted by FACS using mitochondrial specific dyes, reprogrammed by retrovirus and quantified based on GFP positive colonies, indicating endogenous activation of the pluripotent marker Oct-4. Fibroblasts of low mitochondrial activity exhibited 5-fold greater Oct-4 activation compared to highly active cells and 3-fold increases versus non-sorted controls. However, analysis of iPSC clones demonstrated reduced stability of the ES-like state in low versus high activity clones. Current work is investigating underlying mechanisms and will help define a role for mitochondria in pluripotency and reprogramming.

D

An N-terminal truncated carboxypeptidase E splice isoform induces metastasis by activating nedd9 and other metastasis inducing genes.

Saravana R.K. Murthy, Terence K. Lee, Niamh X. Cawley and Y. Peng Loh

Cancer mortality is often from metastatic disease rather than the direct effect of the primary tumor. Identification of metastasis inducing genes may offer valuable mechanistic insight for guiding specific therapeutic strategies. We report here that the carboxypeptidase E gene (CPE) is alternatively spliced in human tumors to yield an N-terminal truncated protein (CPE-DELTA-N) that drives metastasis. CPE-DELTA-N mRNA was elevated in human metastatic colon, breast and HCC cell lines. Suppression of CPE-DELTA-N expression in these cell lines by si-RNA significantly inhibited their growth and invasion. To confirm these observations in vivo, an orthotopic nude mouse model was established. The mice implanted with a tumor derived from HCC cells transfected with si- CPE-DELTA-N RNA in the liver did not show tumor growth or metastasis, compared to scrambled controls. In HCC cytosolic CPE-DELTA-N protein was translocated to the nucleus and upregulated the expression of neural precursor cell expressed, developmentally downregulated gene 9 (Nedd9), through interaction with histone deacetylase (HDAC) 1/2. Inhibition of HDAC activity by the HDAC inhibitors suppressed expression of NEDD9, without effecting CPE-DELTA-N expression. The enhanced invasive phenotype of HCC cells stably transfected with CPE-DELTA-N was suppressed when Nedd9 was silenced by si-RNA. cDNA Microarray studies of HCC cells overexpressing CPE-DELTA-N showed elevated expression of 27 genes associated with metastasis such as Nedd9, claudin 2 (cldn2), carcinoembryonic antigen-related cell adhesion molecule 5 (ceacam5), matrix metallopeptidase 1 (mmp1), plasminogen activator (plat) and inositol 1,4,5-trisphosphate 3-kinase A (itpka), while 30 genes associated with tumor suppressor function, which included insulin-like growth factor binding protein 5 and 3 (igfbp5 and igfbp3) and h19 were down-regulated. These data were further confirmed by qRTPCR. Robust increase in the levels of protranscriptional acetylated histone H3 and H4 in these cells suggest epigenetic regulation of downstream genes by CPE-DELTA-N. Thus CPE-DELTA-N induces tumor metastasis by regulating NEDD9 and other metastasis associated genes expression via interaction with HDAC complex. In clinical studies of 14 patients with thyroid papillary carcinoma, resected tumors having very high copy numbers of CPE-DELTA-N mRNA was correlated with metastasis. Thus CPE-DELTA-N may be a potentially useful biomarker for diagnosing metastasis.

E

Expression Profiling of Autism Candidate Genes during Normal Human Brain Development Implicates Central Immune Signaling Pathways

Ziats MN, Rennert OM

The Autism Spectrum Disorders (ASDs) are neurodevelopmental diseases with heritability estimates over 90 percent in identical twins. Despite substantial efforts to uncover the genetic basis of ASD,a clear understanding of the molecular mechanisms underlying Autism remains elusive. No study has yet characterized the expression profile of all genes implicated in ASD during human brain development. We hypothesized that focusing gene-network analysis on highly expressed genes may provide novel insight into central pathways in ASD.

We devised a novel computational approach that is the first to describe the normal brain expression profile of all ASD genes. We parsed the NIMH Transcriptional Atlas of Human Brain Development for all 219 genes cataloged to date. The NIMH atlas contains next-generation RNA sequencing data from 16 brain regions spanning 21 weeks gestation through 23 years. We then analyzed differential expression across regions and time, and assessed cell-type specific expression using the Human Protein Atlas. Lastly, we discovered novel molecular interaction networks using an enriched set of highly expressed genes.

Remarkably, we found that only 15 percent of genes show high expression in at least one region of the developing brain. Interestingly, the cerebellum--previously implicated in ASD--contained the greatest number of highly expressed genes. At the protein level, 25 percent of highly expressed genes are mainly detected in glia not neurons. We then investigated pathways and networks using this enriched set of highly expressed genes. Gene Ontology revealed two new significant processes-- immune system regulation and apoptosis. Ingenuity Pathway Analysis (IPA), a database of gene-gene interactions, suggested many new pathways not otherwise implicated, including neutrophil signaling. Most significantly, IPA network analysis placed the highly expressed gene network at the center of all networks calculated using all ASD genes. In this central network NFkB, Jnk, and MAPK are hubs--further implicating immune system signaling as a potential final common pathway.

In summary, we describe differential expression of all ASD candidate genes in the developing brain. Gene enrichment implicates central immune signaling pathways at multiple levels of analysis, and suggests glia--in addition to neurons--deserve consideration. This work will be an important resource in Autism research, and provides evidence for a role of the immune system in ASD.

Hindered diffusion in polymeric solutions studied by Fluorescence Correlation Spectroscopy

Silviya Zustiak, Ralph Nossal, Dan Sackett

The typical cell is crowded with both charged and neutral molecules which hinder solute diffusion and ultimately impact all cellular processes. For example, crowding has been shown to trigger aggregation of the amyloidogeneic proteins associated with neurotoxicity in Alzheimer's disease. Despite the vast interest in the subject, hindered diffusion in the cell is still poorly understood. In particular, the combined effect of crowding and non-specific binding has rarely been addressed, partly due to the inability to decouple their individual contributions in the heterogeneous cell environment.

We developed a homogeneous in-vitro cell model, with tunable binding and crowding, in order to elucidate their relative roles in solute diffusion. We used Fluorescence Correlation Spectroscopy (FCS) to measure the diffusion of a charged protein, Ribonuclease A (RNase), in dextran solutions of various charges (acting as binders) and concentrations (acting as crowders). Originally designed for measurements in dilute media, FCS has recently been employed in studies of diffusion in crowded media. However, such diffusion is mainly interpreted as anomalous, with little insight into the specific causes for the anomaly. We used a more complex two-component FCS analysis, which allowed us to extract quantitative binding data. The non-specific nature of the binding was confirmed by incremental addition of salt, which prevented binding by shielding the RNase and dextran charges. Moreover, we validated our FCS results by a separate, well established technique - ultrafiltration.

In agreement with existing data, we observed an overall 5 fold decrease in RNase diffusivity at the highest concentration of dextran, where binding accounted for 75% and crowding for 25% of the decrease. Interestingly, binding decreased RNase diffusivity by 32% even at 0.4 μM dextran. In contrast, crowding affected diffusivity only above a crowder concentration of 20 μM. Further analysis revealed that 100 μM crowder, as compared to 1 μM binder, was needed to achieve equivalent reduction in RNase diffusivity. However, the data suggested that at a higher crowder concentration (300 μM,similar to that in the cell), crowding would overpower the effect of binding.

This is the first study to highlight the relative contribution of non-specific binding and crowding to hindered diffusion in crowded and charged media and thus can facilitate future understanding of molecular transport implicated in key cellular processes.

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